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Estimation of genetic divergence among maize genotypes under high plant densities and late sowing dates using cluster analysis

Entidhar Rashid Mirza[1] **Banan Hassan Hadi**[2]

[1,2]**Crops Sciences Department, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq.**

Corresponding author: b.h.hadi@coagri.uobaghdad.edu.iq

Abstract:

With the aim of grouping similar Egyptian and Iraqi genotypes of maize into homogeneous groups according to sowing date and plant density using cluster analysis and estimating the genetic divergence of the studied genotypes, the hierarchical clustering technique was used. A field experiment was carried out at Research Station A of the College of Agricultural Engineering Sciences - University of Baghdad, Department of Field Crops, in the Al-Jadriya area during the autumn season of 2023. A randomized complete block design (RCBD) was used with a split spilt-plots arrangement with four replications and three factors. The main plots included the two sowing dates (1st August and 10th), and the sub plots included the plant densities (70.000 and 90.000 plants ha^{-1}). Six genotypes of maize, two Iraqi and four Egyptian (Baghdad3, Al-Maha, TW-78, TW-345, IY-355, and IY-207), represented the sub-sub plots. The cluster analysis results showed that the genotypes differed in their clustering patterns according to sowing date and plant density. Euclidean distances also varied depending on the clustering pattern. The two genotypes that exhibited significant Euclidean distances were due to genetic differences and the presence of favorable genes not found in the other studied genotypes. The cluster analysis revealed that the Euclidean distance between the Iraqi variety Baghdad 3 and the Egyptian variety IY-355 was 8.779 and 14.47 at the first sowing date and densities of 70.000 plants h^{-1}and 90.000 plants h^{-1}, respectively. In contrast, the Iraqi variety Baghdad 3 and the Egyptian variety TW-78 exhibited an Euclidean distance of 9.44 at the second sowing date and densities of 70,000 plants h^{-1}. At the second sowing date and densities, the same Iraqi variety and the Egyptian variety TW-345 exhibited the highest Euclidean distance of 9.58. Therefore, it is recommended to include these varieties in crosses with the genotypes that have shown positive results. It is clear that variation is used to take advantage of the hybrid vigor and isolations from it, and thus we conclude that this statistical technique is a successful alternative to molecular techniques in case they are not available.

Keywords: *Maize, sowing dates, plant densities, Cluster analysis.*

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تقدير التباعد الوراثي بين التراكيب الوراثية للذرة الصفراء تحت الكثافات النباتية العالية ومواعيد الزراعة المتأخرة باستخدام التحليل العنقودي

انتظار راشد مرزة[1] بنان حسن هادي [2]

[1,2]قسم علوم المحاصيل، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق.

الخلاصة

بهدف تجميع التراكيب الوراثية المتشابهة من الذرة الصفراء المصرية والعراقية ضمن مجاميع متجانسة وفقاً لموعد الزراعة والكثافة النباتية باستخدام التحليل العنقودي، وتقدير التباعد الوراثي للتراكيب الوراثية المدروسة، استُخدمت تقنية التحليل العنقودي الهرمي. نُفذت تجربة حقلية في محطة البحوث (A) التابعة لكلية علوم الهندسة الزراعية – جامعة بغداد، قسم المحاصيل الحقلية، في منطقة الجادرية خلال الموسم الخريفي لعام 2023. استُخدم تصميم القطاعات العشوائية الكاملة (RCBD)بترتيب القطع المنشقة – المنشقة (Split–Split Plot) وبأربعة مكررات وثلاثة عوامل. شملت القطع الرئيسية موعدي زراعة (الأول من آب والعاشر من آب)، في حين شملت القطع الثانوية الكثافتين النباتيتين (70,000 و90,000 نبات هـ⁻¹). مثّلت ستة تراكيب وراثية من الذرة الصفراء، اثنان عراقيان وأربعة مصرية(Baghdad3 ، Al-Maha، TW-78، TW-345، IY-355، و(IY-207)، القطع الثانوية الثانوية. أظهرت نتائج التحليل العنقودي أن التراكيب الوراثية اختلفت في أنماط تجميعها تبعاً لموعد الزراعة والكثافة النباتية، كما تباينت المسافات الإقليدية بحسب نمط التجميع. وقد عُزي ظهور مسافات إقليدية معنوية بين بعض التراكيب الوراثية إلى الاختلافات الوراثية ووجود جينات مرغوبة غير متوافرة في التراكيب الوراثية الأخرى المدروسة. بيّن التحليل العنقودي أن المسافة الإقليدية بين الصنف العراقي بغداد 3 والصنف المصري IY-355بلغت 8.779 و14.47 عند موعد الزراعة الأول وبكثافتي 70,000 نبات هـ⁻¹ و90,000 نبات هـ⁻¹ على التوالي. في المقابل، أظهر الصنف العراقي بغداد 3 والصنف المصري TW-78 مسافة إقليدية مقدارها 9.44 عند موعد الزراعة الثاني وبكثافة 70,000 نبات هـ⁻¹. وعند موعد الزراعة الثاني وبالكثافات النباتية، أظهر الصنف العراقي نفسه مع الصنف المصري TW-345أعلى مسافة إقليدية بلغت 9.58. وبناءً على ذلك، يُوصى بإدخال هذه الأصناف في برامج التهجين مع التراكيب الوراثية التي أظهرت نتائج إيجابية. ويتضح أن استغلال التباين يسهم في الاستفادة من قوة الهجين وما ينتج عنها من انعزالات، وبالتالي يُستنتج أن هذه التقنية الإحصائية تُعد بديلاً ناجحاً للتقنيات الجزيئية في حال عدم توفرها.

الكلمات المفتاحية: *الذرة الصفراء، مواعيد الزراعة، الكثافات النباتية، التحليل العنقودي.*

1. Introduction

The Maize crop is of great importance due to its nutritional and industrial value, as it contains a high percentage of oils, proteins, and carbohydrates. In addition, it is used in animal feed as green fodder or concentrated feed (Al-Baidhani et al., 2022). Cluster analysis is considered a statistical method that can be applied to data. It is an effective tool for estimating genetic diversity among genotypes and identifying the locations of quantitative traits (QTLs), as well as not requiring assumptions about data distribution (Yau and Ortize, 1994). Cluster analysis is sometimes used to classify maize entries, and it can be used by breeders and geneticists to identify subgroups of entries that are useful for breeding purposes or specific genetic objectives (Rincon et al., 1996). Cluster analysis is referred to as Segmentation and Taxonomy analysis and involves identifying similar groups within a set of cases (genotypes) for specific traits based on the similarity of their responses to surrounding environmental conditions and classifying genotypes according to their genetic closeness or distance (Hamdalla, 2011). There is a significant gap between Iraq's productivity rate and global production, which may be due to the improper utilization of growth inputs in a way that affects maize productivity, such as the optimal planting time and plant density. The main goal of using cluster analysis in plant breeding experiments is to group genotypes into several homogeneous groups so that these genotypes within the group have a similar response pattern across sites (Shrestha, 2016). Alzubaidy et al. (2017) concluded When studying 9 maize inbred lines CA21R, Th97Alla-K122, ZM49R, Inbreed12, G105, G54, ZM47R, ZM51, and G17, the highest genetic distance was 0.141 between the two pure inbred lines G17 and ZM51, which is due to their different genetic material, and the lowest genetic distance was 0.001 between the tester CA21R and the inbred line. This is a type of statistical method that can be applied to the data. Cluster analysis is an effective tool for estimating genetic diversity among genotypes and identifying the locations of quantitative traits (QTL), and it does not require any assumptions about the distribution of the data (Yau and Ortize, 1994). Cluster analysis, also called Segmentation and Taxonomy, examines the identification of similar groups of a set of cases(genotypes) for specific traits based on the similarity of their responses to surrounding environmental conditions and classifies the genetic structures according to their genetic closeness or divergence (Hamdalla, 2011). Based on genetic distance, the genetic relationship among the pure inbred lines was identified in clusters. The tested inbred lines were distributed into three main groups: the first included inbred line G17, the second included ZM47R and Th97Alla-K122 along with the tester ZM47R and Inbred line12, while the third group included G105 and G54. The researchers indicated the presence of genetic differences between the inbred lines. Alguboury and Jumaa (2018) studied seven pure lines of maize (OH40, ZP-301, UNUu052, SH, IK-58, R-153, and IK8) and their half-sibling hybrids, along with a commercial hybrid for comparison. The cluster analysis results indicated that there were two main groups: the first group included four genotypes, 1, 2, 4, and 7, while the second group consisted of two secondary groups. The first secondary group contained genotypes 3 and 6, and the second secondary group contained a single genotype, 5. The highest genetic distance was between the parents IK8 and OH40, at 11,870.75, while the lowest genetic distance was between the parents IK8 and IK-58, at 260.53. The researchers mentioned that it is possible to benefit from genetically distant parents to improve local genotype by transferring their superior genes through breeding methods. Hassan et al. (2019) indicated, of their study on individual hybrids resulting from the crossing of 5 pure inbred lines, that the groups were divided into two sets. The first group included the hybrids ZM49W3E×ZM60, ZM49W3E×ZM43WIZE, ZM49W3E×CDCN5, ZM60×ZM43WIZE, ZM60×CDCN5, ZM43WIZE×ZM19, and ZM19×CDCN5, while the second group included the hybrids ZM49W3E×ZM19, ZM60×ZM19, and ZM43WIZE×CDCN5. This study was conducted to estimate the genetic divergence between the genes of introduced Egyptian and Iraqi genotypes of maize, as indicated by plant yield trait. The studied genotypes were then grouped according to sowing date and plant density for use as parent in breeding programs. The study also aimed to highlight the importance of cluster analysis and its potential as an alternative to molecular analyses, which are relatively expensive or unavailable.

2. Materials and Methods

A field experiment was conducted at the A Research Station of the College of Agricultural Engineering Sciences - University of Baghdad - Department of Field Crops in the Jadriya area during the 2023 fall season to study cluster analysis of Egyptian and Iraqi maize genotypes under late planting dates and high plant densities.

Table (1): Names and symbols of maize Genotypes used in the study and their source.

Number of Genotypes	Genotypes symbols	Source
1	Baghdad 3	Ministry of Agriculture - Department of Agricultural Research, Iraq
2	Al-Maha	
3	TW-78	Al-Azhar University, college of Agriculture, Egypt
4	TW-345	

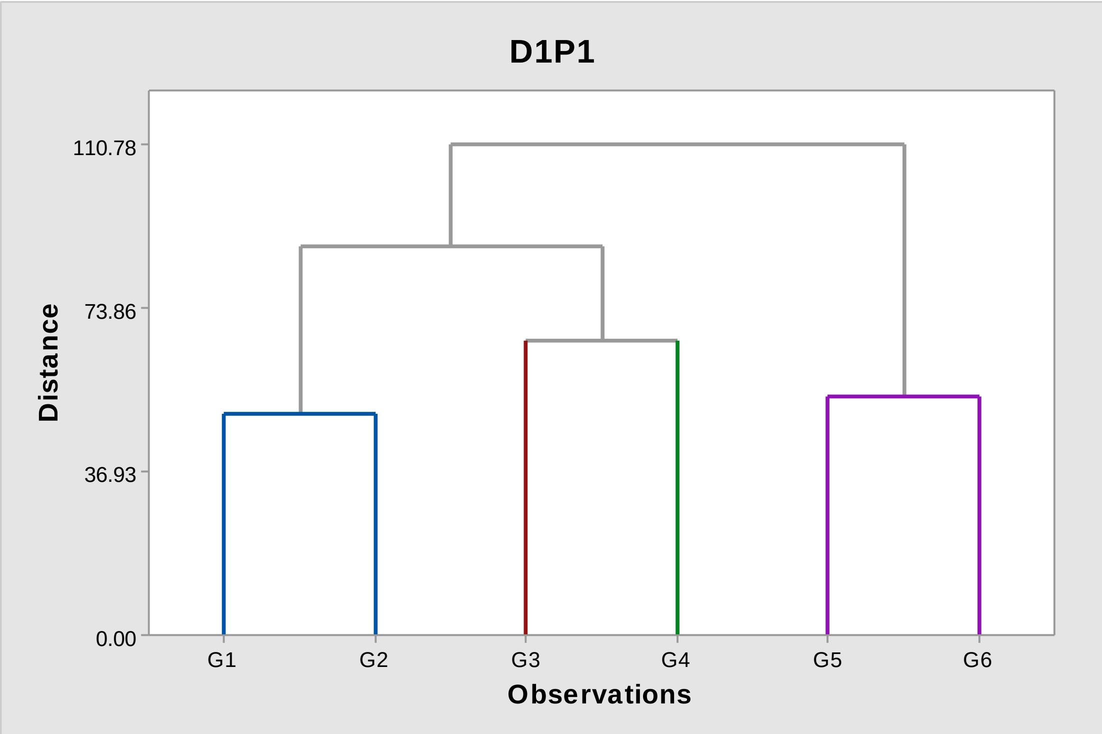
5	IY-355	
6	IY-207	

The study included three factors: the first factor involves two planting dates, on the 1st and 10th of August; the second factor involves two plant densities (70,000 and 90,000 plants per hectare); and the third factor includes six maize genotypes, four of which are Egyptian (labeled TW-78, TW-345, IY-355, IY-207) and two locals (Baghdad-3 and Al-Maha). The experiment was designed as a randomized complete block design (RCBD) with split-split plot arrangement, where the main plots included the planting dates, the subplots included plant densities, and the sub-subplots included the genotypes. The agricultural land was prepared by performing all the necessary farming operations, including plowing, smoothing, and leveling. The soil was fertilized with triple superphosphate at a rate of 200 kg P_2O_5 ha^{-1} in a single application before planting (Saleh and Salman, 2005). The land was divided into 96 plots, each representing an experimental unit, with each plot measuring (2×3 m²) . Each plot included the planting of four rows of maize, with a spacing of 75 cm between rows and 14.8 cm between plants for a plant density of 90,000 plants ha^{-1}, and 19.04 cm between plants for a plant density of 70,000 plants ha^{-1}. The seeds were sown at a rate of 2-3 seeds per hole, and the plants were thinned to one plant per hole. Nitrogen fertilizer was applied at a recommended rate of 350 kg N ha^{-1} in the form of urea (46% nitrogen) in three doses: the first dose two weeks after planting, the second at the elongation stage, and the third at the flowering stage (Saleh and Salman, 2005). Weeding was carried out several times throughout the growing season, and irrigation was done whenever necessary. Cluster Analysis was conducted using SPSS software to determine the degree of divergence among the genetic structures used for each plant density and each planting date. The following traits were studied: days to 50% tassling (days), days to 50% silking (days), plant height (cm), main ear height (cm), stem diameter (cm), number of leaves (leaves per plant), leaf area (m²). The leaf length under the main spike leaf (Lb) was measured for five plants randomly for each experimental unit, and the leaf area was estimated using the formulas established by Elsahookie, 1985, as follows: The leaf area of a plant with 10-12 active leaves = $(\text{leaf length})^2 \times 0.64$, and the leaf area of a plant with 13-15 active leaves = $(\text{leaf length})^2 \times 0.75$. Leaf area index: The leaf area index was estimated using the following equation: Leaf Area Index = Leaf Area / Area Occupied by One Plant. Yield traits and components include: ear length (cm), number of ear rows (rows per year), number of grains per row (grains per row), number of grains per ear (grains per year), 100-grain weight (g), ear diameter (cm), ear weight (g), total dry matter (g), days to 95% physiological maturity (days), number of ears per plant (ears per plant), crop growth rate, individual plant yield (g), unit area yield (tons ha^{-1}), and harvest index (%).

3. Results and Discussion

3.1. Cluster Analysis of Genetic Structures at the Date of August 1 and Plant Density of 70,000 Plants ha^{-1}

Figure (1) shows the distribution of the studied genotypes into three groups based on cluster analysis. The first group included the two genotypes Baghdad 3 and Al-Maha, the second group included two genotypes TW-78 and TW-345, and the third group included two genotypes IY-355 and IY-207. The distribution of the studied genotypes into different groups indicates the presence of genetic divergence among them. Table (2) refers to the stages of forming the cluster pattern, beginning with the first stage by merging the Baghdad 3 genotypes with the Al-Maha genetic structure into a single group, referred to as the Baghdad 3 or 1 group, depending on the smaller sequence of the two genotypes that formed this group. The selection of these two genotypes was due to them having the smallest distance, which was 4.986. In the second stage, the genotypes IY-355 was combined with the genotypes IY-207 based on the distance value between them, which amounted to 5.378, thus forming a new group called Group IY-355 or 5. In the third stage, the genotype TW-78 was combined with the genotype TW-345 into a single group called Group TW-78 or 3, with the distance between them being 6.643. In the fourth stage, Group 1 (Baghdad 3) was merged with Group 3 (TW-78) into a single group called Group 1 or Baghdad 3, with a distance between them of 8.779. In the final stage (fifth), Group 1 (Baghdad 3) was merged with Group 5 (IY-355) into a new group called Baghdad 3 or 1, which had the greatest distance of 11.078. The increase in distance indicates a lack of similarity between the groups, which suggests that the studied genotypes were dissimilar from each other. This result is consistent with the findings of Alguboury and Jumaa (2018).



G1 refers to Baghdad 3, G2 to Al Maha, G3 to TW-78, G4 to TW-345, G5 to IY-355, and G6 to IY-207.

Figure (1): Hierarchical clustering of the distribution of Maize genotypes across groups (vertical axis) and distances (horizontal axis) for all traits studied on August 1 at a plant density of 70.000 plants per ha^{-1}.

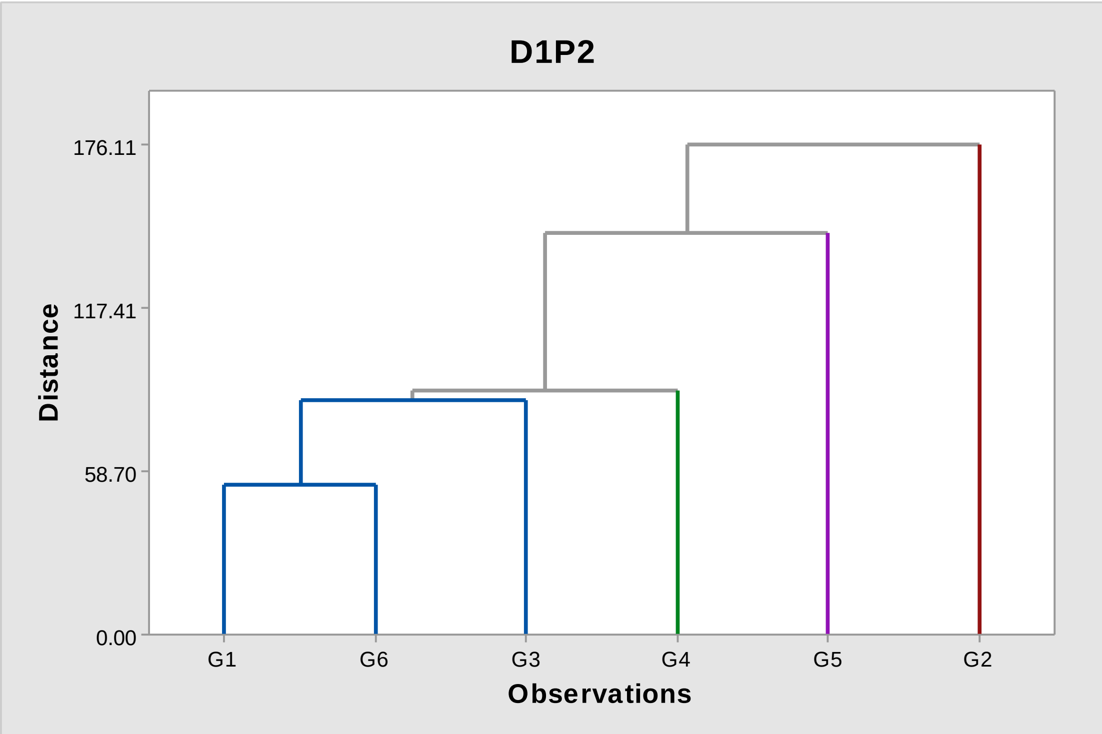
Table (2): Cluster analysis of genotypes at the date of August 1 and plant density of 70.000 plants per ha^{-1}.

Stage	linked clusters	Degree of similarity	Distance
1	1→2	54.98	4.986
2	5→6	51.45	5.378
3	3→4	40.03	6.643
4	1→3	20.75	8.779
5	1→5	0.00	11.078

3.2. Cluster analysis of genotypes on August 1st, with a plant density of 90,000 plants. ha^{-1}.

Figure (2) shows the distribution of the studied genotypes into five groups based on cluster analysis. The first group included the Baghdad 3 and IY-207 genotypes, and differed from the

TW-78, TW-345, IY-355, and Al-Maha genotypes. Each of these genotypes occupied a different group, indicating genetic divergence among them. The second group included the TW-78 genotype alone, the third group included the TW-345 genotype alone, the fourth group included the IY-355 genotype alone, and the fifth group included the Al-Maha genotype alone. Table (3) illustrates the stages of cluster formation. The first stage begins by combining the Baghdad 3 genotype with the IY-207 genotype into a single cluster called Baghdad 3 or 1, depending on the shorter sequence of the two genotypes that formed this cluster.



G1 refers to Baghdad 3, G2 to Al Maha, G3 to TW-78, G4 to TW-345,
G5 to IY-355, and G6 to IY-207.

Figure (2): The hierarchical clustering of the distribution of genotypes of Maize on groups (vertical axis) and distances (horizontal axis) for all studied traits at the date of August 1 and the plant density of 90,000 plants ha^{-1}.

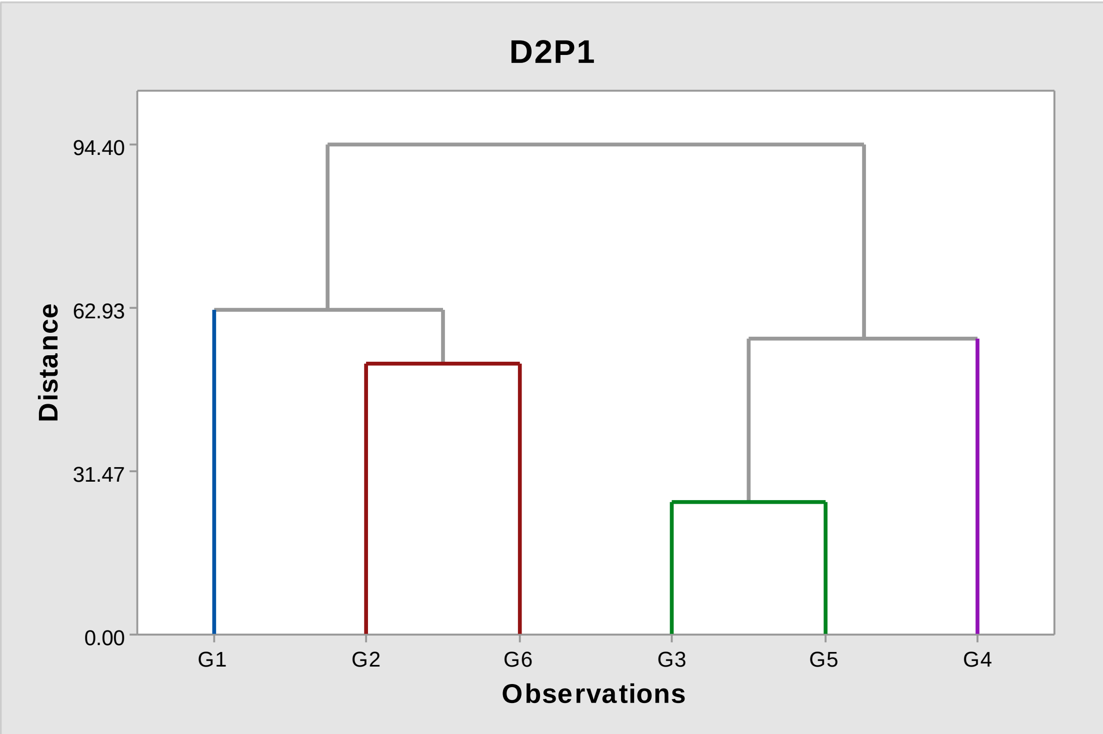
These two genotypes were chosen because they had the smallest distance, which was 5.392. In the second stage, cluster 1 (Baghdad 3) was combined with the TW-78 genotype into a single cluster called Baghdad 3 or 1 (depending on the shorter sequence), and the distance between them was 8.469. In the third stage, cluster 1 (Baghdad 3) was combined with the TW-345 genotype based on the value of the distance between them, which was 8.790, thus forming a new cluster called Baghdad 3 or 1. In the fourth stage, cluster 1 (Baghdad 3) was combined with the IY-355 genotype to form a new cluster called Baghdad 3 or 1, with a distance between them of 14.475. In the fifth (and final) stage, group 1 (Baghdad 3) was merged with the oryx genotype, with a distance of 17.611. This group was named Baghdad 3 or 1. The last genotype to merge is the one furthest from the others and has the greatest distance. This small distance indicates a close relationship between these genotypes. Alzubaidy et al. (2017) reached similar results

Table (3): Cluster analysis of genotypes at the date of August 1 and plant density of 90,000 plants ha^{-1}.

Stage	linked clusters	Degree of similarity	Distance
1	1→6	69.381	5.392
2	1→3	51.909	8.469
3	1→4	50.083	8.790
4	1→5	17.804	14.475
5	1→2	0.000	17.611

3.3. Cluster analysis of genotypes was performed on August 10th at a plant density of 70.000 plants per ha^{-1}.

Figure (3) illustrates the distribution of the studied genotypes into four groups based on cluster analysis. The first group included two genotypes, TW-78 and IY-355. The second group included two genotypes, Al-Maha and IY-207, respectively. The third group included the TW-345 genotype alone, while the fourth group included the Baghdad 3 genotype alone. The division of the studied genotypes into distinct groups confirms the genetic divergence between them.



G1 refers to Baghdad 3, G2 to Al Maha, G3 to TW-78, G4 to TW-345, G5 to IY-355, and G6 to IY-207.

Figure (3): The hierarchical clustering of the distribution of genotypes of Maize on groups (vertical axis) and distances (horizontal axis) for all studied traits at the date of August 10 and the plant density of 70.000 plants ha^{-1}.

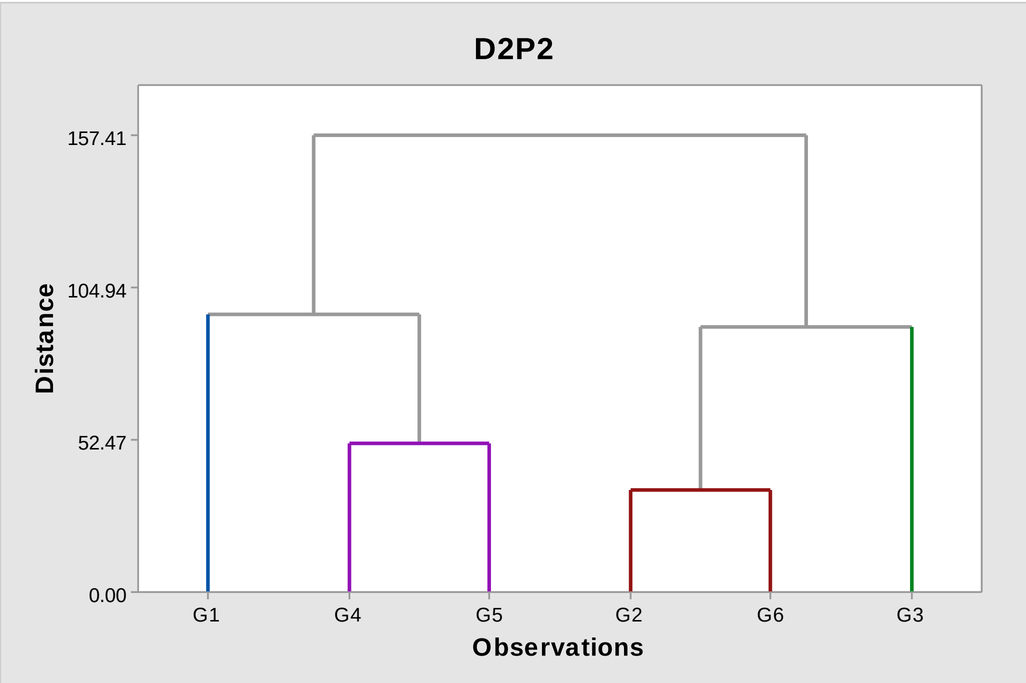
Table (4) shows the stages of cluster formation. It begins with the first stage, in which the TW-78 and IY-355 genotypes were combined into a single group called TW-78 or 3, depending on the shorter sequence of the two genotypes that formed this group. These two genotypes were chosen because they had the shortest distance, at 2.560. In the second stage, the oryx genotype and the IY-207 genotype were combined into a single group called the oryx group or 2, with a distance of 5.223 between them. In the third stage, group 3 (TW-78) was combined with the TW-345 genotype based on their distance value of 5.723, thus forming a new group called TW-78 or 3 (according to the shorter sequence). Then, in the fourth stage, the Baghdad 3 and Group 2 (Al-Maha) genotypes were combined to form a new group called Baghdad 3 or 1, with a distance of 6.252. In the final (fifth) stage, Group 1 (Baghdad 3) and Group 3 (TW-78) were combined into a single group called Baghdad 3 or 1, with a distance of 9.440, the greatest distance between these two groups. The increasing distance between the groups indicates their differences. This result confirmed the findings of Hassan et al. (2019).

Table (4): Cluster analysis of genotypes on August 10 and plant density of 70.000 plants ha^{-1}.

Stage	linked clusters	Degree of similarity	Distance
1	3→5	72.877	2.560
2	2→6	44.667	5.223
3	3→4	39.366	5.723
4	1→2	33.767	6.252
5	1→3	0.000	9.440

3.4. Cluster analysis of genotypes on August 10th, with a plant density of 90.000 plants. ha^{-1}.

Figure (4) shows the distribution of genotypes into four groups. The first group included two genotypes (Al-Maha and IY-207), while the second group included two genotypes, TW-345 and IY-355.



G1 refers to Baghdad 3, G2 to Al Maha, G3 to TW-78, G4 to TW-345, G5 to IY-355, and G6 to IY-207.

Figure (4): The hierarchical clustering of the distribution of genotypes of Maize on groups (vertical axis) and distances (horizontal axis) for all studied traits at the date of August 10 and the plant density of 90,000 plants ha^{-1}.

The third group included the genotype TW-78 alone, and the fourth group included the genotype Baghdad 3 alone. Table (5) illustrates the stages of cluster formation. The first stage begins with the merging of the oryx genotype and the IY-207 genotype into a single group called the oryx group or 2 (depending on the shorter sequence). These two genotypes had the smallest distance, at 3.500. In the second stage, the TW-345 genotype was merged with the IY-355 genotype to form a new group called the TW-345 or 4 group, with a distance of 5.116. In the third stage, group 2 (Al-Maha) was merged with the TW-78 genotype into a single group called the Al-Maha group or 2 (depending on the shorter sequence), with a distance of 9.168. In the fourth stage, the Baghdad 3 genotype and group 4 (TW-345) were merged to form a new group called the Baghdad 3 or 1 group, with a distance of 9.586. In the fifth stage, Group 1 (Baghdad 3) was merged with Group 2 (Al Maha) into one group called Group Baghdad 3 or 1, and these two groups recorded the longest distance of 15,740. This result was similar to the result Hamdall (2011).

Table (5): Cluster analysis of genetic structures on August 10 and plant density of 90.000 plants ha^{-1}.

Stage	linked clusters	Degree of similarity	Distance
1	2→6	77.764	3.500
2	4→5	67.498	5.116
3	2→3	41.755	9.168
4	1→4	39.096	9.586
5	1→2	0.000	15.740

4. Conclusions and Recommendation

The clustering of the maize introduced genotypes differed with the Iraqi varieties depending on the sowing date and plant density. The Iraqi variety Baghdad 3 showed the greatest divergence from the Egyptian varieties at high plant densities and late sowing dates. Possessing distinct and genetically distinct genotypes is fundamental to the success of plant breeding and improvement programs. Furthermore, determining genetic diversity increases the efficiency of breeding programs, particularly hybridization between parents carrying the favorable genes. The use of cluster analysis is very useful for estimating genetic diversity when molecular techniques are unavailable. Therefore, we recommend using this technique for Egyptian and Iraqi genotypes in .saline or arid environments, or in multiple locations

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