**Study of Genetic Divergence and Enzymatic Specialization of Maize Inbred Lines**

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**Abstract**

An experiment field was carried out in the field of the College of Agricultural Engineering Sciences, University of Baghdad during spring season of 2019 to studying the genetic divergence and enzymatic specialization of six inbred lines of maize (Zai 6, Zai 7, Zai 8, Zai 9, Zai 14 and Zai 19). The experiment was conducted according to RCBD with four replications. The results showed that the Zai 6 inbred line was significantly outperformed in the POD, SOD and CAT enzymes activity (48.44, 73.50 and 27.89 unit g-1) respectively and plant content of proline (2.216 µmol g-1), while the Zai 8 was significantly outperformed in the plant height (197.5 cm), and the Zai 9 was significantly outperformed in the leaf area (5268 cm2), leaves of chlorophyll (44.97 µg g-1 fresh weight), plant dry weight (357 g plant-1), number of grains per ear (609.9 grain ear-1) and grain yield (7.829 t ha-1). The results of the cluster analysis showed that the Zai 6 and Zai 8 inbred lines were the most genetic divergence, as the distance between them reached 7.67629. We can conclude that the Zai 6 and Zai 8 inbred lines can be used in a commercial hybrid production by using the phenomenon of genetic divergence in increasing hybrid vigor.

**Keywords:** *Zea mays* L., **a**ntioxidant enzymes, cluster analysis, genetic variation.

**Introduction**

Environmental changes and pests are the biggest challenges facing agricultural production (Adhab *et al*. 2018), because of their direct impact on the physiological and biochemical processes of crops and ultimately lead to a decrease in grain yield and quality (Miller *et al*. 2010), as the exposure of the plant to any biotic or abiotic stress affects in photosynthesis and the water, carbon, nutritional and hormonal balance within its tissues, as well as its role in the accumulation of free oxygen species (ROS) that destroy biological molecules of cells, which negatively affects the products of photosynthesis, plant growth and productivity (Adhab *et al*. 2019).

Accordingly, the cultivation of seeds of plants that can tolerance various abiotic stresses is an effective biological measure to mitigate the harmful effects of unsuitable environmental conditions (Najem and Hamza 2023), and at the same time it can produce environmental and economic benefits, which enhances the sustainable development of agriculture (Mahajan and Tuteja 2005), and that the breeding of germplasm resistant to these stresses and taking advantage of the available resources have an important role in maintaining crop productivity and yield stability, which ensures food security (Wang *et al*. 2022).

The content of plants of antioxidant enzymes such as SOD, CAT, POD, and APX is one of the means by which the harmful effect of any environmental stress can be reduced as a result of its sequential and simultaneous action in scavenging of free radicals, as well as being one of the tools by which the ability of inbred lines to resist environmental conditions can be predicted, and then the possibility of developing hybrids and cultivars that are tolerant to the biotic and abiotic stresses that the crop is expected to be exposed to during its life cycle (Mao *et al*. 2011).

The use of new genetic material in breeding programs is necessary, but it must be of a genetic divergence different from the local genotypes to ensure that they possess new genes that don't exist in their counterparts (Elsahookie and Abed 2008). Cluster analysis can be used to assess genetic divergence because it is an effective method to examine the genetic purity and determine the genetic groups according to the different genotypes (Abed 2011; Hassan *et al*. 2019).

Therefore, this experiment was carried out to studying the enzymatic specialization and genetic divergence of maize inbred lines to provide the theoretical basis through which commercial hybrids of maize can be developed that are resistant to unfavorable environmental conditions.

**Materials and Methods**

**Experimental details and treatments**

An experiment field was carried out in the field of the College of Agricultural Engineering Sciences - University of Baghdad during spring season of 2019 to studying the genetic divergence and enzymatic specialization of six inbred lines of maize (Zai 6, Zai 7, Zai 8, Zai 9, Zai 14 and Zai 19). The experiment was conducted according to RCBD with four replications.

Soil management were carried out, and then the experiment land was divided into 24 experimental units, each experimental unit included 5 lines, 75 cm apart, and 25 cm between hills to reach a plant density of 53333 plants ha-1. Nitrogen fertilizer was added with an average of 400 kg N ha-1 as a urea (46% N) at two equal doses, the first after 20 days of emergence, while the second after 30 days of the first dose (Cheyed and Elsahookie 2011). The seeds of maize inbred lines were sown on the 1 March 2019 by 3 seeds per hill, and then thinned to one seedling after emergence. Crop management was carried out as needed, and the plants were harvested after the appearance of maturity signs.

**Studied traits**

The peroxidase (POD),Superoxide dismutase (SOD) and Catalase (CAT) enzymes activity were measurement at a 100% flowering stage according to Nezih (1985); Sarkar *et al*. (2001) and Sairam and Srivastava (2001) methods respectively. The plant content of proline (µmol g-1) was measurement at a 100% flowering stage according to Bates *et al*. (1973) method. In addition to, the plant height (cm), leaves number per plant, leaf area (cm2): (leaf length under leaf ear)2 x 0.65 (Elsahookie 1985), leaves content of chlorophyll (µg g-1 fresh weight) and dry weight of plant (g plant-1) were measurement at a 100% flowering stage, while the grains number per ear, 500 grains weight (g) and grain yield (t ha-1) were measurement at a harvest stage.

**Statistical analysis**

The data were statistically analyzed using SPSS program and Duncan multiple test at 0.05 probability level was used to comparison between averages (Steel and Torrie 1960), while the genetic divergence was analyzed using Spare 2 program**.**

**Results**

**Peroxidase (POD) enzyme activity (unit g-1)**

The results in Table 1 indicate that the Zai 6 inbred line was significantly outperformed and recorded a highest average of POD enzyme activity (48.44 unit g-1) with non-significant difference with Zai 7 inbred line (45.72 unit g-1) compared to Zai 9 inbred line which recorded a lowest average of POD enzyme activity (33.88 unit g-1) with non-significant difference with Zai 8 and Zai 14 inbred lines (35.21 and 37.39 unit g-1) respectively.

**Superoxide dismutase (SOD) enzyme activity (unit g-1)**

The results in Table 1 show that the Zai 6 inbred line was significantly outperformed and gave a highest average of SOD enzyme activity (73.50 unit g-1) with non-significant difference with Zai 7 and Zai 8 inbred lines (71.45 and 68.54 unit g-1) respectively compared to Zai 14 inbred line which gave a lowest average of SOD enzyme activity (57.29 unit g-1) with non-significant difference with Zai 9 inbred line (59.16 unit g-1).

**Catalase (CAT) enzyme activity (unit g-1)**

The results in Table 1 reveal that the Zai 6 inbred line was significantly outperformed and achieved a highest average of CAT enzyme activity (27.89 unit g-1) with non-significant difference with Zai 7 inbred line (26.12 unit g-1) compared to Zai 9 inbred line which achieved a lowest average of CAT enzyme activity (20.05 unit g-1) with non-significant difference with Zai 14 inbred line (22.31 unit g-1). The difference of maize inbred lines in the production of antioxidant enzymes may be due to the difference in their genetic nature in the ability of their genes to produce the enzymes that responsible for the effectiveness of metabolic processes related with plant growth in the environmental conditions in which they were grown (Abed *et al*. 2018; Jessup *et al*. 2020; Wang *et al*. 2022).

**Plant content of proline (µmol g-1)**

According to research data, The results in Table 1 indicate that the Zai 6 inbred line was significantly outperformed and recorded a highest average of plant content of proline (2.216 µmol g-1) with non-significant difference with Zai 7 and Zai 8 inbred lines (2.080 and 1.665 µmol g-1) respectively compared to Zai 9 inbred line which recorded a lowest average of this trait (0.817 µmol g-1) with non-significant difference with Zai 19 inbred line (1.212 µmol g-1).

**Table 1. The difference of maize inbred lines difference in the enzymatic specialization and proline content**

|  |  |  |
| --- | --- | --- |
| **Plant content of proline (µmol g-1)** | **Enzymes activity (unit g-1)** | **Inbred lines** |
| **CAT** | **SOD** | **POD** |
| 2.216 a | 27.89 a | 73.50 a | 48.44 a | **Zai 6** |
| 2.080 ab | 26.12 ab | 71.45 a | 45.72 a | **Zai 7** |
| 1.665 abc | 24.29 bc | 68.54 ab | 35.21 bc | **Zai 8** |
| 0.817 d | 20.05 d | 59.16 c | 33.88 c | **Zai 9** |
| 1.482 bc | 22.31 cd | 57.29 c | 37.39 bc | **Zai 14** |
| 1.212 cd | 23.13 c | 64.50 b | 39.31 b | **Zai 19** |

**Plant height (cm)**

The results in Table 2 show that the Zai 8 inbred line was significantly outperformed and gave a highest average of plant height (197.5 cm) with non-significant difference with Zai 19, Zai 9 and Zai 6 inbred lines (190.0, 185.0 and 182.5 cm) respectively compared to Zai 7 inbred line which gave a lowest average of this trait (167.5 cm) with non-significant difference with Zai 9, Zai 6 and Zai 14 inbred lines (185.0, 182.5 and 172.5 cm). The difference of maize inbred lines in this trait could be attributed to the structural differences in the genes controlling the plant height, which appear in the form of phenotypic behavior expressed by the variations between the inbred lines in this trait, as the derived inbred lines differ in their genetic nature according to the parents resulting from them and the method of breeding used, which increases the genetic variation between the offspring resulting from them, which causes differences in metabolic pathways as well as the activity of ROS enzymes (Abed 2011; Al-Temimi and Abed 2016).

**Number of leaves per plant**

The results in Table 2 reveal that there was non-significant difference between maize inbred lines in the number of leaves per plant.

**Leaf area (cm2)**

The results in Table 2 indicate that the Zai 9 inbred line was significantly outperformed and recorded a highest average of leaf area (5268 cm2) with non-significant difference with Zai 14 and Zai 8 inbred lines (4941 and 4537 cm2) respectively compared to Zai 6 inbred line which recorded a lowest average of this trait (3126 cm2) with non-significant difference with Zai 7 inbred line (3672 cm2). The reason of variation in the leaf area of the maize inbred lines may be due to their different genetic nature and their response to environmental conditions.

**Leaves content of chlorophyll (µg g-1 fresh weight)**

The results in Table 2 reveal that the Zai 9 inbred line was significantly outperformed and achieved a highest average of leaves content of chlorophyll (44.97 µg g-1 fresh weight) with non-significant difference with Zai 14 and Zai 8 inbred lines (44.29 and 42.29 µg g-1 fresh weight) respectively compared to Zai 6 inbred line which achieved a lowest average of this trait (35.03 µg g-1 fresh weight) with non-significant difference with Zai 7 inbred line (38.30 µg g-1 fresh weight). The reason of superiority of Zai 9 inbred line in this trait may be due to its superiority in the leaf area (Table 2), and a positive significant correlation between the two traits confirms this result (Table 4), or the difference in the content of leaves of chlorophyll of the maize inbred lines could be attributed to the difference in gene expression of the gene responsible for stay-green in these inbred lines (Okab and Abed 2022).

**Plant dry weight (g plant-1)**

The Zai 9 inbred line was significantly outperformed and recorded a highest average of plant dry weight (375 g plant-1) with non-significant difference with Zai 14 and Zai 8 inbred lines (321 and 295 g plant-1) respectively compared to Zai 6 inbred line which recorded a lowest average of this trait (184 g plant-1) with non-significant difference with Zai 19 and Zai 7 inbred lines (270 and 239 g plant-1) respectively (Table 2). The reason of increase the plant dry weight of Zai 9 inbred line could be duo to decrease the POD, SOD and CAT enzymes activity and their content of proline (Table 1), in addition to superiority in the leaf area, leaves content of chlorophyll (Table 2), which led to an increase the efficiency of the photosynthesis process and an increase its metabolites, and then an increase the accumulation of dry matter in the plant, as the inbred lines differ in the photosynthesis process according to the metabolic pathways that occur in the plant cell (Elsahookie and Abed 2008). The results in Table 4 show that the plant dry weight was a negative significant correlation with the POD, SOD and CAT enzymes activity and plant content of proline, while it was positive significant correlation with leaf area and leaves content chlorophyll (Okab and Abed 2023)

**Table 2. The difference of maize inbred lines in the growth traits**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Plant dry weight (g plant-1)** | **Leaves content of chlorophyll (µg g-1 FW)** | **Leaf area (cm2)** | **No. leaves per plant** | **Plant height (cm)** | **Inbred lines** |
| 184 c | 35.03 d | 3126 d | 13.50 a | 182.5 abc | **Zai 6** |
| 239 bc | 38.30 cd | 3672 cd | 12.25 a | 167.5 c | **Zai 7** |
| 295 ab | 42.29 ab | 4537 abc | 14.25 a | 197.5 a | **Zai 8** |
| 375 a | 44.97 a | 5268 a | 13.25 a | 185.0 abc | **Zai 9** |
| 321 ab | 44.29 a | 4941 ab | 13.25 a | 172.5 bc | **Zai 14** |
| 270 bc | 39.41 bc | 4153 bc | 13.75 a | 190.0 ab | **Zai 19** |

**Number of grains per ear**

Regarding of number of grains per ear, the results in Table 3 reveal that the Zai 9 inbred line was significantly outperformed and achieved a highest average of grains number per ear (609.9 grain ear-1) with non-significant difference with Zai 8 and Zai 14 inbred lines (592.9 and 568.7 grain ear-1) respectively compared to Zai 7 inbred line which achieved a lowest average of this trait (475.4 grain ear-1) with non-significant difference with Zai 6 inbred line (490.8 grain ear-1). The reason of increase the number of grains per ear in Zai 9 inbred line may be due to its recording the lowest results of POD, SOD and CAT enzymes activity and plant content of proline (Table 1), as well as its recording the highest results of leaf area, leaves content of chlorophyll and plant dry weight (Table 2), which led to an increase the conversion of photosynthesis products to the reproductive parts, and then an increase the percentage of fertilized florets, which positively reflected on the number of grains in the ear, as it appears from the results of Table 4 that the number of grains per ear was negatively significant correlation with the POD, SOD and CAT enzymes activity and plant content of proline and it was positively significant correlation with leaf area, leaves content of chlorophyll and plant dry weight (Abed *et al*. 2018; Okab and Abed 2023).

**500 grains weight (g)**

The results in Table 3 reveal that there was non-significant difference between maize inbred lines in the 500 grains weight.

**Grain yield (ton ha-1)**

The results in Table 3 indicate that the Zai 9 inbred line was significantly outperformed and achieved a highest average of grain yield (7.829 t ha-1) with non-significant difference with Zai 8 and Zai 14 inbred lines (7.611 and 7.300 t ha-1) respectively compared to Zai 7 inbred line which achieved a lowest average of grain yield (6.103 t ha-1) with non-significant difference with Zai 6 inbred line (6.301 t ha-1). The reason of superiority of Zai 9 inbred line in this trait may be due to its superiority in the number of grains per ear (Table 3), and a positive significant correlation between the two traits confirms this result (Table 4).

**Table 3. The difference of maize inbred lines in the grain yield and its components**

|  |  |  |  |
| --- | --- | --- | --- |
| **Grain yield (t ha-1)** | **500 grains weight (g)** | **No. grains per ear** | **Inbred lines** |
| 6.301 bc | 122.19 a | 490.8 c | **Zai 6** |
| 6.103 c | 121.82 a | 475.4 c | **Zai 7** |
| 7.611 a | 121.36 a | 592.9 a | **Zai 8** |
| 7.829 a | 120.11 a | 609.9 a | **Zai 9** |
| 7.300 a | 123.26 a | 568.7 ab | **Zai 14** |
| 6.771 b | 125.44 a | 535.2 b | **Zai 19** |

**Table 4. Correlation coefficient between studied factors**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Trait** | **X1** | **X2** | **X3** | **X4** | **X5** | **X6** | **X7** | **X8** | **X9** | **X10** | **X11** |
| **X1** | **-----** |  |  |  |  |  |  |  |  |  |  |
| **X2** | **0.6737** | **-----** |  |  |  |  |  |  |  |  |  |
| **X3** | **0.7586** | **0.8933** | **-----** |  |  |  |  |  |  |  |  |
| **X4** | **0.8397** | **0.6330** | **0.6805** | **-----** |  |  |  |  |  |  |  |
| **X5** | **-0.3705n.s** | **0.0986n.s** | **-0.0503n.s** | **-0.3638n.s** | **-----** |  |  |  |  |  |  |
| **X6** | **-0.2122n.s** | **-0.1557n.s** | **-0.1524n.s** | **-0.1065n.s** | **0.4604** | **-----** |  |  |  |  |  |
| **X7** | **-0.6963** | **-0.7285** | **-0.7656** | **-0.5443** | **0.1303n.s** | **0.1537n.s** | **-----** |  |  |  |  |
| **X8** | **-0.7269** | **-0.6932** | **-0.6518** | **-0.5800** | **0.1475n.s** | **-0.0341n.s** | **0.7539** | **-----** |  |  |  |
| **X9** | **-0.5874** | **-0.6946** | **-0.7060** | **-0.4286** | **-0.0014n.s** | **0.1287n.s** | **0.9327** | **0.7151** | **-----** |  |  |
| **X10** | **-0.7179** | **-0.4123** | **-0.4836** | **-0.5389** | **0.3191n.s** | **0.1127n.s** | **0.6182** | **0.6021** | **0.5485** | **-----** |  |
| **X11** | **0.1091n.s** | **-0.0284n.s** | **0.0232n.s** | **0.1298n.s** | **0.0145n.s** | **0.4331** | **-0.0610n.s** | **-0.2259n.s** | **0.0158n.s** | **-0.0576n.s** | **-----** |
| **X12** | **-0.7025** | **-0.4161** | **-0.4848** | **-0.5123** | **0.3049n.s** | **0.1045n.s** | **0.6125** | **0.6180** | **0.5432** | **0.9940** | **-0.0624n.s** |
|  |
| **X1 = POD** | **X2 = SOD** | **X3 = CAT** | **X4 = Plant content of proline** | **X5 = Plant height** | **X6 = No. leaves per plant** |
| **X7 = Leaf area** | **X8 = Leaves content of chlorophyll** | **X9 = Plant dry weight** | **X10 = No. grains per ear** | **X11 = 500 grains weight** | **X12 = Grain yield** |

**Cluster analysis of maize inbred lines**

The results in Table 5 indicate the distribution of the studied maize inbred lines into five groups based on cluster analysis. The results in Figure 1 and Table 5 show the stages of cluster formation. The first stage merging of Zai 6 inbred line with Zai 7 inbred line into one group called Group 1 or Zai 6 inbred line depending on the lesser sequence of the two inbred lines that made up this group and the reason of selecting these two inbred lines was due to their possessing of the least distance (2.86672). In the second stage, combining Zai 9 inbred line with the Zai 14 inbred line occurred based on value of the distance between them, which amounted to 2.98537, to form a new group called group 2 or Zai 9. In the third stage, the combination of Zai 8 inbred line with Zai 19 inbred line in a group called group 3 or Zai 8 with the distance between them at 3.32352. Afterward, the group 2 was merged with group 3 to form a new group called group 4 (Zai 8 inbred line), as the distance between them reached 4.79189. The last merger was between group 4 and group 1, which has the farthest distance of 7.67629. The final merged inbred lines were the farthest from the rest of inbred lines and had the broadest distance. The lake of distance reveals the close relationship between these inbred lines. Therefore, avoiding hybridization between them (Ali and Alshugeairy 2023). Elsahookie and Abed (2008) reported that the increasing of the genetic divergence between the maize inbred lines led to an increase the hybrid vigor and specific combing ability (SCA), which results in an increase the effect of the dominant gene action on the traits of the resulting hybrid. The longest calculated distance between Zai 6 and Zai 8 inbred lines reached to 7.67629, which can refer to the difference in the genetic origin of these two inbred lines, also it means the presence of large polymorphism, which indicates the possibility of using these two inbred lines in producing a commercial hybrid by exploiting the phenomenon of genetic divergence in increasing the hybrid vigor (Hamzah *et al*. 2013; Al-Mehemdi and Abed 2016).

**Table 5. Cluster analysis of maize inbred lines**

|  |  |  |  |
| --- | --- | --- | --- |
| **Stage** | **Associated cluster** | **Degree of similarity** | **Distance** |
| **1** | **Zai 6 Zai 7** | **62.6549** | **2.86672** |
| **2** |  **Zai 9 Zai 14** | **61.1092** | **2.98537** |
| **3** |  **Zai 8 Zai 19** | **56.7041** | **3.32352** |
| **4** | **Zai 8 Zai 9** | **37.5754**  | **4.79189** |
| **5** | **Zai 6 Zai 8** | **0.0000** | **7.67629** |

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**Figure 1. Hierarchical grouping of the distribution of maize inbred lines on the totals (vertical axis) and distances (horizontal axis) for all the studied traits**

**Conclusion**

According to the research data, we conclude that the Zai 6 inbred line was significantly outperformed in the activity POD, SOD and CAT enzymes and plant content of proline, whereas the Zai 9 was significantly outperformed in the leaf area, leaves of chlorophyll, plant dry weight, number of grains per ear and grain yield. Also, the cluster analysis results showed that the Zai 6 and Zai 8 inbred lines were the most genetic divergence, so we can conclude that the Zai 6 and Zai 8 inbred lines can be used in a commercial hybrid production by using the phenomenon of genetic divergence in increasing hybrid vigor.

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**Author Contributions**

ESN planned the experiments and conducted field work; IAH conducted lab works; ZAA reviewed final manuscript; and AAM statistically analyzed the data and interpreted the results.

**Conflicts of Interest**

All authors declare no conflict of interest.

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

**Ethics Approval**

Not applicable to this paper.

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