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Study of activity antioxidant enzymes in several of maize genotypes

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ABSTRACT

INTRODUCTION. Iraq is suffering from severe drought, exacerbated by climate change. Genetic patterns and timing of planting are of critical importance to improve water use efficiency and sustainability of agricultural production.

MATERIALS AND METHODS. A field experiment was conducted at the Al-Jadriya field, Baghdad University, College of Agricultural Engineering Sciences. Six maize genotypes (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880, and G6-IK58) were planted on three dates (1 August, 7 August, and 15 August) during the fall season of 2023–2024. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Leaf samples were analyzed in the laboratories of the Department of Field Crops, College of Agricultural Engineering Sciences. The least significant difference (LSD) test at the 0.05% probability level was used for mean comparisons, and statistical analysis was performed using the GenStat statistical software.

RESULTS. The results indicated that genotype G6 exhibited higher catalase (CAT) activity across all three planting dates, while genotype G2 showed higher peroxidase (POD) activity during the same period, suggesting superior adaptive efficiency of these two genotypes. Superoxide dismutase (SOD) activity also varied among genotypes, with the highest levels observed on D3 (third planting date) and D1 (first planting date) for G6, and on D2 (second planting date) for G5, indicating a response due to genotype × planting date (G × D) interaction. Regarding planting date, CAT activity was highest on D3, whereas POD and SOD activities peaked on D1, reflecting the influence of planting date on the regulation of the antioxidant defense system.

CONCLUSION. The results demonstrate that enzyme activity and maize response to planting dates vary significantly depending on genotype. Therefore, selecting an appropriate planting date is essential for optimizing crop growth under specific light, humidity, and temperature conditions.

KEYWORDS: *Zea mays* L., antioxidant enzymes, planting dates, genotype, enzyme activity, high temperature https://doi.org/10.31279/ABNC-2026-16-3-138

Изучение активности антиоксидантных ферментов у различных генотипов кукурузы

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АННОТАЦИЯ

ВВЕДЕНИЕ. Ирак испытывает серьезный дефицит влаги, усугубляемый изменением климата. Генетические особенности и сроки посева имеют решающее значение для повышения эффективности использования воды и устойчивости сельскохозяйственного производства.

МАТЕРИАЛЫ И МЕТОДЫ. Полевой опыт был проведен на опытном поле Аль-Джадрия при Багдадском университете, Колледж инженерных наук в сельском хозяйстве. Шесть генотипов кукурузы (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880 и G6-IK58) высевали в три срока (1 августа, 7 августа и 15 августа) в осенний сезон 2023–2024 гг. Опыт закладывали по схеме рандомизированных полных блоков в трехкратной повторности. Образцы листьев анализировали в лабораториях кафедры полевых культур Колледжа инженерных наук в сельском хозяйстве. Для сравнения средних значений использовали тест наименьшей существенной разности при уровне вероятности 0,05 %, статистическую обработку данных выполняли с помощью программы GenStat.

РЕЗУЛЬТАТЫ. Результаты показали, что генотип G6 характеризовался более высокой активностью каталазы во все три срока посева, тогда как генотип G2 проявлял повышенную активность пероксидазы в те же периоды, что свидетельствует о высокой адаптивной эффективности этих двух генотипов. Активность супероксиддисмутазы (СОД) также различалась между генотипами: наибольшие значения отмечены у генотипа G6 в третий (D3) и первый (D1) сроки посева, а у генотипа G5 — во второй срок (D2), что указывает на зависимость ферментативного ответа от взаимодействия «генотип × срок посева». Что касается сроков посева, активность каталазы была максимальной в D3, тогда как активности пероксидазы и СОД достигали пика в D1, что отражает влияние срока посева на регуляцию эффективности антиоксидантной системы защиты.

ЗАКЛЮЧЕНИЕ. Полученные результаты демонстрируют, что активность ферментов и реакция кукурузы на сроки посева существенно варьируют в зависимости от генотипа. Таким образом, выбор оптимального срока посева имеет важное значение для оптимизации роста культуры в конкретных условиях освещенности, влажности и температуры.

КЛЮЧЕВЫЕ СЛОВА: кукуруза *Zea mays* L., антиоксидантные ферменты, сроки посева, генотип, ферментативная активность, высокая температура

INTRODUCTION

Zea mays L. is the third most important cereal crop in the world after wheat and rice, due to its wide nutritional and economic importance [1]. Despite their high production capacity, they are classified as crops that are relatively sensitive to water stress, especially drought, which may negatively affect plant growth, development, and productive components [2]. Therefore, determining the appropriate planting date is an important agricultural procedure that should be taken into consideration, ensuring that the sensitive stages of plant growth are compatible with the appropriate environmental and climatic conditions, and reducing the crop's exposure to environmental stresses [3]. Early detection of the genetic behavior of varieties under different environmental conditions is of importance in determining the extent of response of varieties and selecting the best [4].

Global warming and increased temperatures and droughts, especially in Iraq, are taken as a serious risk to the growth of plants and productivity. Determining the appropriate planting date is at the forefront of the factors affecting the success of the production process, as it is directly linked to the different stages of plant growth, which are affected by the availability of appropriate environmental conditions, especially temperature, humidity, lighting intensity, and length of the light period.

Antioxidant enzymes are one of the most important components of the physiological defense system in *Zea mays* L. plants, as they play a pivotal role in maintaining oxidative balance and protecting cells from damage resulting from the accumulation of reactive oxygen species under conditions of environmental stress, especially drought and high temperatures. The variation in the efficacy and activity of SOD, CAT, and POD among *Zea mays* L. cultivars may reflect their different ability to cope with oxidative stress and adapt to adverse environmental conditions, making these enzymes important physiological indicators in assessing cultivar tolerance and selecting the most efficient cultivar under stress conditions.

The antioxidant activity is one of the first lines of defense against oxidation and it is important to maintain an optimal redox environment for oxidation and reduction [5]. It revealed that the efficiency of the antioxidant enzyme system differed significantly among the genotypes and the early planting date had a significant effect on peroxidase activity, whereas the late planting date had the highest activity in both superoxide dismutase and catalase. This is a pattern of very effective complementary gene-environmental interaction [6]. Under normal physiological conditions, NADPH oxides NOXES are one of the major producers of cellular ROS and are a current research interest, as they have a specific ROS-producing role [7]. CAT is a quaternary enzyme which plays an important role in the breakdown of hydrogen peroxide (H_2O_2) to water and oxygen in a highly efficient and effective manner.

The activity of the Catalase enzyme decreased significantly at high temperatures [8] and increased in Catalase enzyme activity during heat stress [9]. Oxidizing enzymes play an important role in plants' response to biotic and abiotic stress [10, 11]. Heat stress resulted in a reduction in the percentage of the enzymatic antioxidants including Catalase CAT, Peroxidase POD and Superoxidase dismutase SOD [12]. It has recently been demonstrated that O_2 accumulation contributes to activation of the superoxidase dismutase enzyme SOD, which neutralizes O_2 to H_2O_2 and is thus one of the antioxidant enzymes that are able to protect corn from oxidative damage, as well as roots [13, 14]. Under high temperature conditions, ROS formation is increased and enzymes like catalase are activated as a defense mechanism but in severe stress conditions enzymes' activity is disrupted [15]. The antioxidant enzymes are the first defense line against oxidative harm and it is essential to maintain the oxidation and reduction environment at the optimum level [16].

The enzyme superoxidase is less active at higher temperatures [11]. When no stress, contents of antioxidant enzymes are increased and must select a proper planting date [5]. Optimal condition for catalase enzyme activity was confirmed which is 37°C [17]. Heat stress can affect the response of oxidative stress enzymes to changes in planting dates [18]. This work focused on the importance of antioxidant enzymes to reduce the stress of plants with heavy metals [19]. The stress can affect the plants negatively impacting growth and yield [20].

This study aims to evaluate the response of several *Zea mays* L. varieties to different planting dates, and to determine the effect of different planting dates on the activity and effectiveness of some oxidative enzymes associated with the plant's response to environmental stresses. It also aims to detect variation between varieties in their ability to adapt to prevailing environmental conditions, and to identify varieties that are most efficient in responding to heat stress and drought, thus contributing to the selection of the best and most suitable varieties for cultivation under Iraq's environmental conditions, and promoting their use in breeding programs, genetic improvement, and sustainable crop production.

Average thermal degrees throughout the growing period (Figure 1) were obtained from the Iraqi weather monitoring agency (2023–2024).

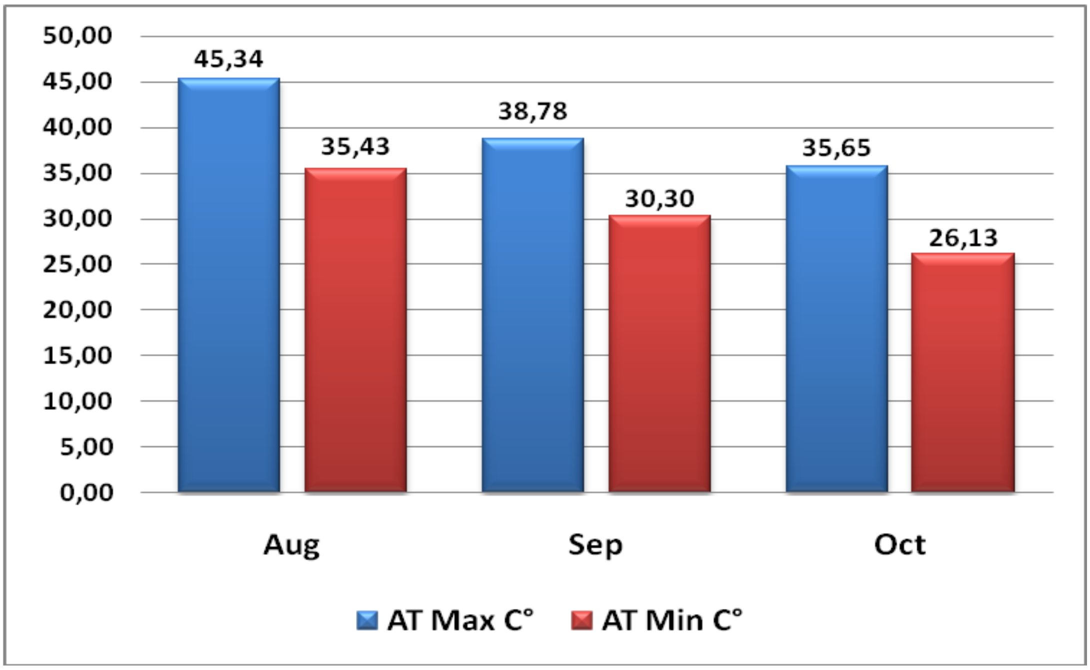

Figure 1
Average maximum (Max) and minimum (Min) temperatures in autumn 2023–2024

MATERIALS AND METHODS

A field experiment was performed in the autumn season (2023–2024) at the fields of Baghdad University College of Agricultural Engineering Sciences for six genotypes of maize (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880 and G6-IK58) on the following planting dates: 1 August, 7 August, and 15 August. A randomized complete block design (RCBD) was employed for the experiment with three replications. The mean squares were calculated using the least significant difference (LSD) 0.05% test was used to compare the GenStat statistical program used for data analysis. Average thermal degrees throughout the growing period were obtained from the Iraqi weather monitoring agency (2023–2024).

Determination of enzyme activity

At the flowering stage, 10 mL of phosphate buffer (KH_2PO_4, pH 7.8) was added to each frozen leaf sample (stored at −25 °C). To estimate enzyme activity, the following enzymes were assayed using a spectrophotometer:

– Determination catalase enzyme activity (CAT)

Enzyme activity was determined in accordance with the experimental method [21].

$$Enzym\ activity\ CAT\ \left(U\backslash mg\ protein\right) = \frac{\Delta\ reading\ \backslash\Delta\ t}{0.1 \times 0.01 \times protein\ concentation},$$

where Δ *reading* – change in absorbance/reading;

Δ *t* – time interval (usually in minutes);

0.1 – volume of enzyme/sample used;

0.01 – path length/correction factor as specified by the assay;

protein concentration – protein concentration of the sample (usually mg/mL).

– Determination of peroxidase enzyme activity (POD)

Peroxidase enzyme activity was estimated according to the method of [22].

$$Enzyme\ activity\ POD\left(\ U\backslash mg\ protein\right) = \frac{\Delta\ reading\ \ \backslash\Delta\ t}{0.1 \times 0.01 \times protein\ concentation}.$$

– Determination of superoxidase dismutase enzyme activity (SOD)

Enzyme activity was determined in accordance with the experimental method [23].

$$SOD\,(inhibition) = \frac{(A2B - A1B) - (A2S - A1S)}{(A2B - A1B)} \times 100,$$

where *A1B* – blank absorbance before lighting;
A2B – blank absorbance after lighting;
A1S – absorbance of the sample before lighting;
A2S – absorbance of the sample after lighting.

$$Enzyme\ activity\ SOD = \frac{sample\ inhibition}{highest\ inhibition} \times \frac{df}{Vs}(U\backslash mL) Measruing\ unit,$$

where *df* – dilution factor;
U – unit;
Vs – sample size.

RESULTS

Catalase enzyme (CAT)

The plant has a complicated redox defense system that responds to the excessive productivity of ROS and shields the plants from the injury caused by ROS, when their production surpasses the activity of antioxidant enzymes like catalase, which causes damage to essential components of the plant. As is observed from results in Figure 2, the highest average protein content across the three planting dates were obtained in G5 with protein content of 2.74, 2.51 and 2.18 unit.mg^{-1} protein for D3, D2, and D1 respectively. The genotype G4 recorded the maximum 2.27 average protein content in D3, G2 genotype recorded the minimum 1.12 average protein in D2 and G1 recorded the minimum 1.19 average protein in D3 while G2 recorded the minimum 1.12 average protein in D2. The genotype G1 in planting date 1 August D1 had average 1.34 unit.mg^{-1} protein.

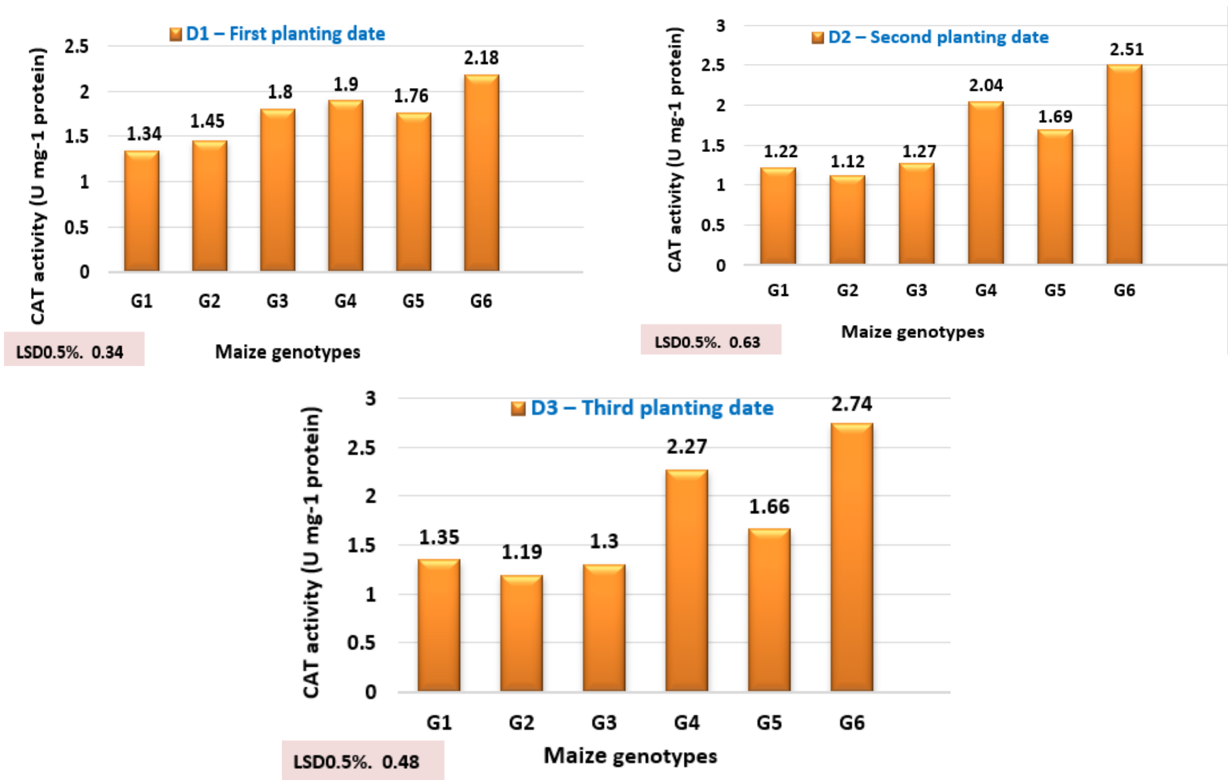

Figure 2

Effect of planting date on Catalase CAT enzyme activity unit.mg^{-1} protein for six genotypes of maize (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880, and G6-IK58) in autumn season 2023–2024

Figure 3 shows that the results differ significantly with regard to planting date: D3 (15 August) gives the highest average activity (1.8 unit mg^{-1} protein), followed by D1 (1 August) with 1.7 unit mg^{-1} protein, while D2 (7 August) gives the lowest average (1.6 unit mg^{-1} protein). The activity of Catalase enzyme decreased (inhibited) which contradicts the results of [26] that concluded with an increasing in Catalase activity with increasing temperature.

Peroxidase enzyme (POD)

The storage of reactive oxygen species ROS and the degree of membrane harm in the atom promotion in response conditions to high-temperature conditions [24]. According to [27] the variation in the enzymatic activity between genotypes is attributed to their genetic specificity. The data presented in Figure 4 reveal large genotype differences at all planting dates. G2 genotype had the highest average protein content in D3, D1, and D2 as 0.75, 0.74, and 0.72 unit.mg^{-1} protein, respectively. The G5 genotype had the highest average protein content on D1 0.63 unit.mg^{-1} protein, followed by G5 genotype on D2 0.58 unit.mg^{-1} protein and G3 genotype on D3 0.55 unit.mg^{-1} protein, while the genotype G4 had the lowest average protein content on D3, D2, and D1 0.35,0.36 and 0.45 unit.mg^{-1} protein respectively.

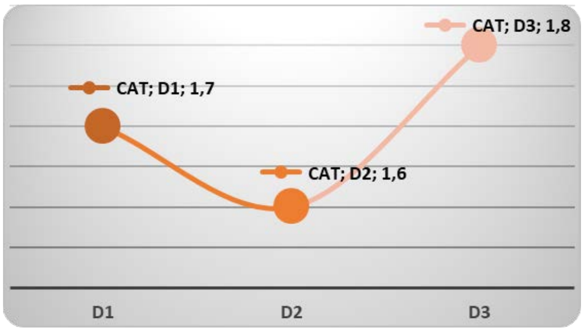

Figure 3
Means of Catalase CAT enzyme activity unit.mg^{-1} protein in three planting dates (1 August D1 first planting date, 7 August D2 second planting date, 15 August D3 third planting date) for six maize genotypes in the autumn season 2023–2024

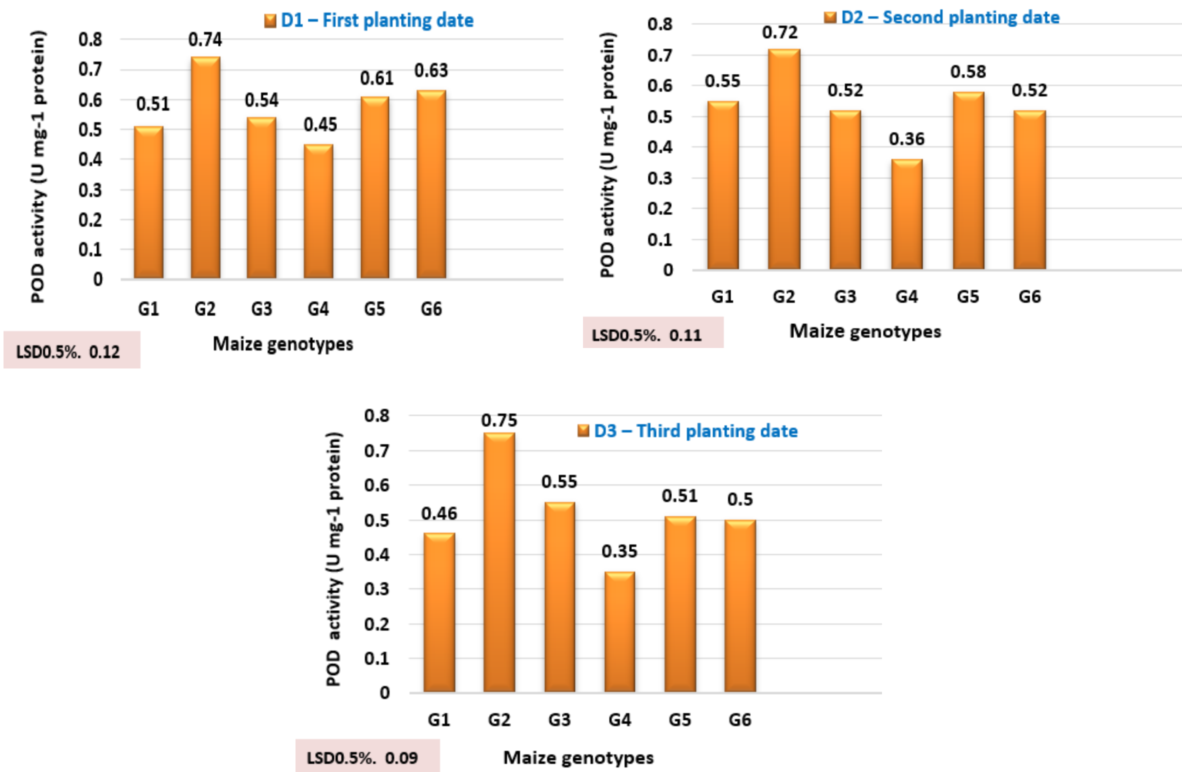

Figure 4
Effect of planting date on Peroxidase POD enzyme activity unit.mg^{-1} protein for six genotypes of maize (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880, and G6-IK58) in autumn season 2023–2024

As shown in Figure 5, high temperatures enhanced the activity of peroxidase enzyme as compared to heat stress with high average on 1 August in D1 with 0.6 unit.mg^{-1} protein, followed by 15 August D3 with an average of 0.52 unit.mg^{-1} protein, while the least average was observed on 7 August in D2 with 0.5 unit.mg^{-1} protein. The results illustrated in Figure 5 revealed that the highest average of enzyme activity was observed on 1 August in D1 with 0.6 unit.mg^{-1} protein, followed by 15 August in D3 with 0.52 unit.mg^{-1} protein, whereas the lowest average was seen on 7 August in D2 with 0.5 unit.mg^{-1} protein. According to [26], enzyme activity goes up as temperature goes up. The results of [11] showed that the values decreased when the temperature increased.

Superoxidase enzyme (SOD)

One of the first defense mechanisms against the superoxide ions O2 in the hydrogen peroxide form H_2O_2 and oxygen O_2 is the action of the superoxide dismutase enzyme [29]. This is because there can be natural resistance to heat stress which could have contributed to the lesser production of ROS mediated by heat stress [30]. Under heat stress situations, levels of ROS and membrane damage in the atom build up [24]. The difference in enzyme activity among genotypes is due to their genetic nature according to [27].

A significant difference between genotypes at each planting date is shown in Figure 6. G5 genotype got the highest average protein content of 64.25 and 55.68 unit.mg^{-1} respectively at D3 and D1. Genotype G5 showed the highest enzyme activity on D2 (51.8 unit.mg^{-1} protein), while genotype G1 had the lowest average enzyme activity across all planting dates, with values of 35.91, 36.12, and 45.91 unit.mg^{-1} protein for D3, D2, and D1, respectively.

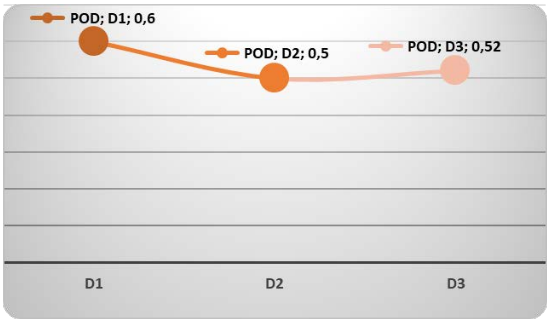

Figure 5
Means of Peroxidase POD enzyme activity unit.mg^{-1} protein in three planting dates (1 August D1 first planting date, 7 August D2 second planting date, 15 August D3 third planting date) for six maize genotypes in the autumn season 2023–2024

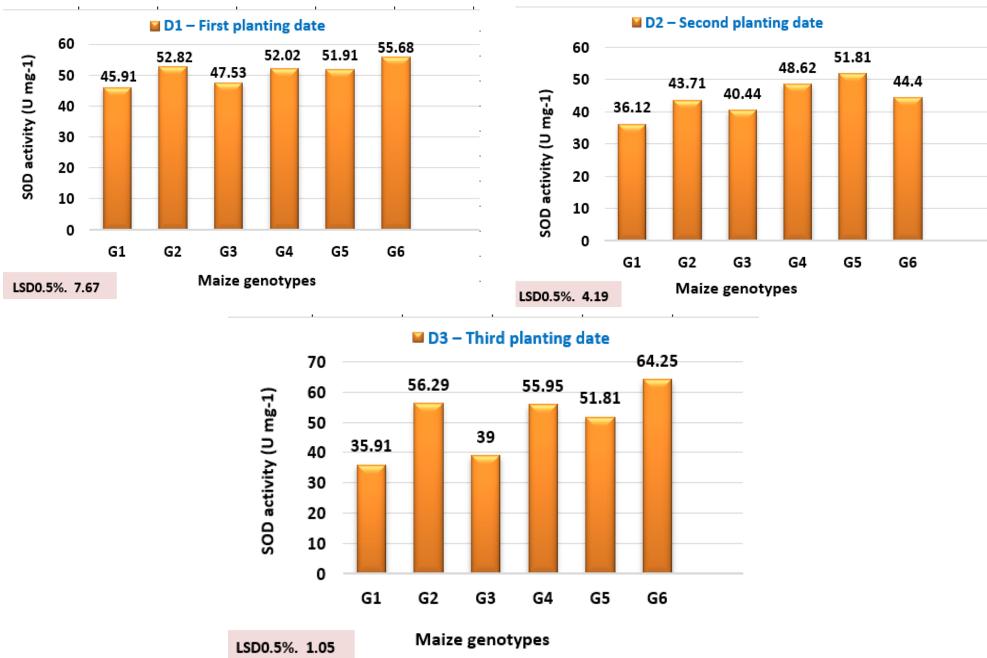

Figure 6
Effect of planting date on Superoxide dismutase (SOD) enzyme activity (unit.mg^{-1} protein) for six maize genotypes (G1-Agr183, G2-PAN466, G3-Ly55, G4-OH40, G5-Xq880, and G6-IK58) during the autumn season of 2023–2024

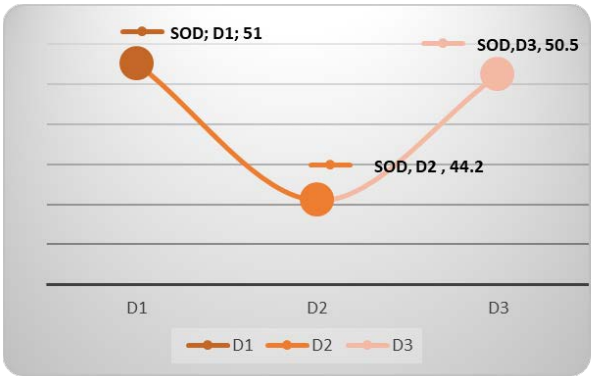

Figure 7
Means of superoxide dismutase SOD enzyme activity (unit.mg^{-1} protein) across three planting dates: D1 (1 August first planting date), D2 (7 August second planting date), and D3 (15 August third planting date) for six maize genotypes in the autumn season of 2023–2024

A variation was found within planting dates (Figure 7). When stressed, plants exhibit stimulation of their redox defense system which looks for Reactive Oxygen Species (ROS). The second date on 7 August showed the lowest average of 44.2 unit.mg^{-1} in D2. Redox defense system contributes to supporting plants to tolerate heat stress [31]. The enzyme activity was increased at lower temperatures, as shown in Figure 1. At high temperatures, the activity of SOD enzyme decreases which is confirmed by [6, 11].

DISCUSSION

The results obtained indicate that the antioxidant system enzyme activities such as CAT, POD and SOD enzyme significantly varied between the different genotypes due to the date of planting, which marked a difference during plant response to heat stress. This difference lies in the genetic efficiency in controlling ROS homeostasis and reducing the toxic effects of ROS on cellular components.

The G5 genotype demonstrated higher activities of CAT and SOD, indicating it is more capable of scavenging ROS and thus better able to withstand heat stress. Some genotypes, like G1 and G2, had lower enzyme activities, suggesting restricted adaptation to high temperatures and leading to high sensitivity to oxidative stress.

Another finding was that POD and SOD were more active at high temperatures linked with certain planting dates, suggesting an adaptive process responsible for removing hydrogen peroxide and free radicals. However, catalase showed a variable response, even inhibition under extreme heat stress, indicating greater sensitivity to heat than other enzymes.

These findings validate the efficiency of the antioxidant system, namely POD and SOD activities as important parameters for heat stress tolerance and highlight the importance of genetic differences in the adaptation of plants to harsh environmental conditions.

CONCLUSION

The results confirm the role of an efficient antioxidant system as a key factor in heat stress tolerance across different genotypes. This high activity of SOD and POD was correlated with efficient ROS scavenging and reduced oxidative damage. The G5 genotype had the highest overall enzyme activity and thus was more heat tolerant than the other genotypes. On the other hand, lower enzyme activity in some genotypes indicates reduced adaptability. In addition, the physiological response is dependent on planting dates, with temperature conditions influencing the activity of certain enzymes, such as catalase, under specific stress conditions.

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