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# Assessing antioxidant system and yield of maize (*Zea mays* L.) inbreds under elevated temperature condition

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## ABSTRACT

Maize (*Zea mays* L.) is the most important food and feed crop grown under diverse soil and climatic conditions. Among the cereals, demand for maize is increasing year after year, but fluctuation in climatic conditions especially the temperature extremes is the current and future threat in maize cultivation. Each degree Celsius increase in global mean temperature causes yield reduction up to 7.4 per cent in maize. The high temperature stress impact at the reproductive stage affects grain filling rate and duration. Adaptation of maize crop to future warmer climatic conditions requires a better understanding of physiological responses to elevated temperatures. With this view, a pot culture experiment was conducted at the Department of Crop Physiology, TNAU, Coimbatore during the summer season of 2020. Two maize inbreds viz., UMI 1230 and CBM-DL- 322 were taken for the study and exposed to high temperature stress treatments viz., T<sub>1</sub>- ambient, T<sub>2</sub>- ambient+4°C and T<sub>3</sub>- ambient+6°C (44°C) for 10 days during the reproductive stage to assess the changes in biochemical and yield traits. The ambient+4°C treatment revealed that the maize inbred line CBM-DL-322 recorded lower malondialdehyde content with over production of antioxidant enzyme activity (superoxide dismutase, catalase and ascorbate peroxidase). Cob weight and seed set parentage showed a negative correlation with both elevated temperatures. It is concluded that the maize inbreds line CBM-DL- 322 performed better at an elevated temperature at ambient+4°C and recorded more cob weight (57.09g) compared to UMI 1230 inbred (43.56g).

**Keywords:** Maize, high temperature, antioxidant enzyme activity, yield.

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## INTRODUCTION

Global climate change leads to adverse effects on agricultural productivity. Among the climate variables, temperature extremes severely affect crop yields when occur during the reproductive stage. Each of the last four decades has been warmer than the previous decade since 1850. According to the IPCC - 2018, if current rates of global warming continue, global temperatures will rise by 1.5 degrees Celsius between 2030 and 2052. Mikhaylov *et al.* (2020) reported that in the last 100 years, the earth's global temperature has increased by 0.5-1 degrees Celsius. According to Raza *et al.* (2019), over the last few centuries, changes in climatic conditions such as temperature and rainfall patterns had a significant impact on the morphology and physiology of a wide range of crop species and so it can generate excess energy in high temperatures, but a larger increase in temperature reduces plant growth and photosynthesis rate.

Maize (*Zea mays* L.) is the thermophilic C<sub>4</sub> plant (Cabral *et al.*, 2017) and one of the world's most important food staples and widely planted cereal (Tefsaye *et al.*, 2017). In India, after rice and wheat, maize is considered as a third important cereal crop grown in a wide range of soil and climatic conditions. According to the Indian maize scenario-2021, in India during 2018-19, maize is cultivated in an area of around 9.2 million ha and the production was 27.8 million MT in 2018-19. Fluctuation in temperature leads to changes in physiology and biochemical aspects of a cell organelle, which accordingly affect the growth and development of maize plants (Sita *et al.*, 2017). The sub-optimal and supra-optimal temperatures have the greatest impact on the reproductive stage of the maize plant (Waqas *et al.*, 2021). Variations in temperature cause cellular membranes damage, prevent nutrient absorption and decrease the activity of many enzymes involved in various metabolic processes of the maize plants (Hussain *et al.*, 2019).

The cell membrane is considered as an important component of cells in the plant and its stability is affected by high temperature,

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due to which leakage of ions from the cells takes place. Plants protect themselves against oxidative stress by enhancing the antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) to scavenge the reactive oxygen species as a consequence of elevated temperatures (Dwivedi *et al.*, 2019). But, the severity of heat stress creates an imbalance between the reactive oxygen species and the antioxidant scavenging system in the cell (Zhu *et al.*, 2010). Thus, membrane stability is affected by various activated oxygen species (AOS) like superoxide anion radical ( $O_2^{\cdot-}$ ), hydroxyl radicals (OH) and hydrogen peroxide ( $H_2O_2$ ) molecules which are produced under high temperature stress (Yuzbasioglu *et al.*, 2017). In maize, at reproductive stage (silking stage), the potential number and size of kernels are defined and grain filling occurs until physiological maturity when the final crop yield is determined. Two weeks before and after silking is a critical period for maize to produce a higher yield. The occurrence of any abiotic and biotic stress during this critical period registers a significant negative impact on yield. Hence, this study was proposed to understand and quantify the impact of elevated temperatures occurring during the reproductive stage on the biochemical and yield traits of selected maize inbreds. In this study, changes in lipid peroxidation (MDA) and antioxidant enzymes activity, cob weight and seed set percentage of yield attributes in maize under elevated temperatures were observed and discussed.

## MATERIAL AND METHODS

### Preparation of Plant Material

Pot culture experiment was carried out during the summer of 2020, at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Pots with a size of 32 cm (diameter) were filled with red soil, clay soil, and vermicompost in a ratio of 2:1:1 for sowing the seeds of maize inbreds (UMI 1230 and CBM-DL-322). Two plants per pot were maintained following a factorial completely randomized design with seven replications. All the recommended practices were followed based on the TNAU crop production guide, to maintain a healthy crop.

### Imposition of High Temperature Stress

The plants were grown in a natural environment until they reached the reproductive stage. At the reproductive stage (52 DAE), maize plants were transferred to a growth chamber (Open Top Chamber-OTC) where the high temperature stress was imposed for 10 days in a closed environment. In the control chamber, the ambient temperature was maintained at 37°C while in OTC, one chamber was maintained at 37°C (ambient) plus 4°C and the other at 37°C plus 6°C. Ten days after the treatment, the stressed plants were moved from the growth chamber to the ambient environment to recover. The observations were taken during stress period at reproductive stage (52 DAE) and plant samples were collected for analysis from each replication and mean values were computed.

### Analysis of Antioxidant System

With slight changes to the technique of measurement, the level of lipid peroxidation in the leaf tissue was quantified

in terms of malondialdehyde concentration determined by the TBA reaction (Hodges *et al.*, 1999). The amount of superoxide dismutase in the leaf sample was measured using the nitroblue tetrazolium (NBT) method outlined by Beauchamp and Fridovich (1971). The activity of ascorbate peroxidase in the leaves was determined using the method of Elavarthi and Martin (2010). Catalase (CAT) activity was assessed as the rate of reduction in hydrogen peroxide and absorbance was taken at 240 nm as suggested Aebi and Bergmeyer (1983).

### Yield Parameters

Finally for yield attributes of cob weight (selected cobs were dried to a moisture content of 12%, weighed and the mean weight was expressed in g) and seed set percentage (seed set percentage = filled/(filled+half filled+unfilled) \* 100%) were determined as described by Prasad *et al.*, (2006). The grains from the sun dried cobs were separated and the average grain weight per plant was expressed in gram per plant.

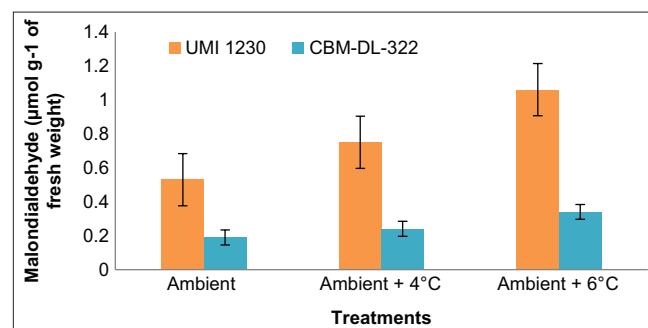
### Statistical Analysis

IBM SPSS Statistics version 23.0 software (<http://www.spss.com>) was used for statistical analysis. The mean values of each character were examined using analysis of variance to determine their significance.

## RESULTS

### Malondialdehyde Content

From the data (Figure 1), it was observed that the UMI 1230 inbred ( $0.78 \mu\text{mol g}^{-1}$  of fresh weight) produced significantly higher MDA compared to CBM-DL-322 inbred ( $0.26 \mu\text{mol g}^{-1}$  of fresh weight). Among different high temperature treatments, ambient +6°C ( $0.70 \mu\text{mol g}^{-1}$  of fresh weight) produced significantly higher MDA compared to the other treatments. The interaction effect between genotype and temperature treatments showed that CBM-DL-322 inbred ( $0.19 \mu\text{mol g}^{-1}$  of fresh weight) under ambient conditions produced significantly lower MDA content compared to all other remaining combinations. The inbred UMI 1230 grown under ambient +6°C ( $1.06 \mu\text{mol g}^{-1}$  of fresh weight) produced significantly higher MDA content compared to ambient and ambient +4°C treatments.



**Figure 1:** Effect of elevated temperature stress on malondialdehyde content in maize leaves at reproductive stage

### Superoxide Dismutase Activity

From Figure 2, it was observed that there was an enhanced production of superoxide dismutase in CBM-DL-322 (1.18 mg protein<sup>-1</sup> min<sup>-1</sup>) than the UMI 1230 (1.02 mg protein<sup>-1</sup> min<sup>-1</sup>) under elevated temperature conditions. Both the inbred lines which were exposed to ambient+6°C (1.25 mg protein<sup>-1</sup> min<sup>-1</sup>) recorded a significant increase in the activity of SOD compared to the other temperature treatments. In both temperature treatments i.e., ambient+4°C and ambient+6°C, the inbred CBM-DL-322 inbred line recorded significantly higher SOD activity compared to UMI 1230. The interaction effect also showed that the inbred line CBM-DL-322 grown under ambient+6°C condition recorded significantly higher SOD activity (1.35 mg protein<sup>-1</sup> min<sup>-1</sup>) compared to all other combinations.

### Ascorbate Peroxidase Activity

From Table 1, it was observed that the maize inbred lines exposed to ambient+6°C temperature (43.51 change in OD at 430 nm g<sup>-1</sup>) and ambient+4°C temperature (41.93 change in OD at 430 nm g<sup>-1</sup>) recorded significantly higher APX activity compared to the lines grown under ambient conditions. Among the maize inbreds, CBM-DL-322 registered the highest APX activity at ambient+4°C (44.05 change in OD at 430 nm g<sup>-1</sup>) and at ambient+6°C (46.49 change in OD at 430 nm g<sup>-1</sup>). The interaction between genotype and treatments showed that the inbred line CBM-DL-322 grown under elevated temperature conditions recorded significantly higher APX activity compared to all other combinations.

### Catalase Activity

Table 2 revealed that the maize inbred CBM-DL-322 recorded significantly higher catalase activity (101.11 unit g<sup>-1</sup> min<sup>-1</sup>) compared to UMI 1230 (95.49 unit g<sup>-1</sup> min<sup>-1</sup>). Irrespective of genotypes, under ambient+6°C maize plants showed significantly higher CAT activity (104.02 μmol g<sup>-1</sup> of fresh weight) compared to the other treatments.

### Cob Weight

The results (Figure 3) revealed that the maize inbred CBM-DL-322 recorded significantly higher cob weight (56.67g) compared to UMI 1230 (43.11g) under elevated temperature conditions. But irrespective of genotypes, the maize plants grown under ambient temperature registered significantly increased cob weight (63.80g) compared to the maize plants grown under elevated temperatures. Also, it was observed that the inbreds grown at ambient+4°C (50.33g) were observed with significantly higher cob weight compared to the inbreds grown at ambient+6°C temperature (35.54g).

### Seed Set Percentage

It was observed that the maize inbred CBM-DL-322 registered a significantly higher mean seed set percentage of 72.99% compared to UMI 1230 line (61.07%). Also this study revealed

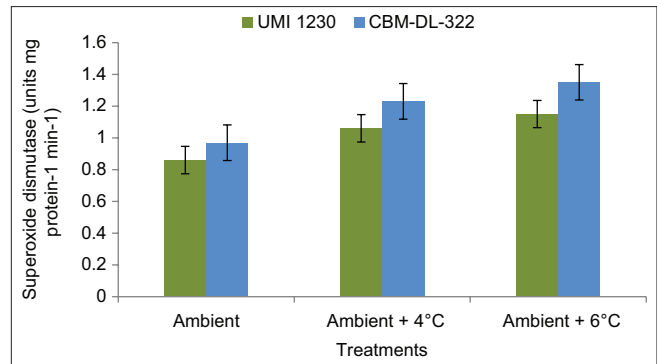


Figure 2: Effect of elevated temperature stress on SOD activity in maize leaves at reproductive stage

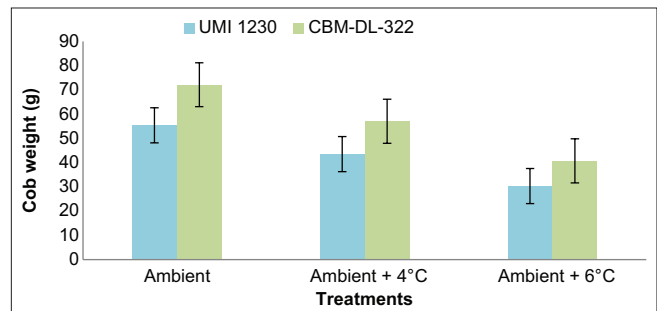


Figure 3: Effect of elevated temperature stress on cob weight (g) in maize inbreds

Table 1: Effect of elevated temperature stress on ascorbate peroxidase (APX) activity in maize leaves at reproductive stage

Treatments/ Genotypes	Ascorbate peroxidase (change in OD at 430 nm g <sup>-1</sup> )		
	UMI 1230	CBM-DL-322	Temperature Mean
Ambient (37°C)	36.25	37.46	36.86
Ambient+4 °C	39.80	44.05	41.93
Ambient+6 °C	40.52	46.49	43.51
Genotype Mean	38.86	42.67	
	Genotype	Treatment	Interaction
CD	1.401	1.716	2.426
SEm	0.486	0.596	0.843

Table 2: Effect of elevated temperature stress on catalase (CAT) activity in maize leaves at reproductive stage

Treatments /Genotypes	Catalase (unit g <sup>-1</sup> min <sup>-1</sup> )		
	UMI 1230	CBM-DL-322	Temperature Mean
Ambient (37°C)	88.85	91.28	90.07
Ambient+4 °C	97.03	104.60	100.82
Ambient+6 °C	100.58	107.45	104.02
Genotype Mean	95.49	101.11	
	Genotype	Treatment	Interaction
CD	3.373	4.131	NS
SEm	1.171	1.434	2.029

that the maize inbreds grown under ambient+4°C temperature (66.54%) recorded significantly higher seed set percentage compared to the inbreds grown under ambient+6°C condition (44.65%). Under ambient+4°C, the inbred CBM-DL-322 showed a significantly higher seed set percentage (77.82%)

than the UMI 1230 (55.25%). But, under ambient+6C, CBM-DL-322 inbred line (49.40%) does not differ significantly with UMI 1230 line (55.25%) (Figure 4).

## DISCUSSION

### Malondialdehyde Content

Malondialdehyde, a product of peroxidation of unsaturated fatty acids, was used as a diagnostic tool for free radical damage to the cell membrane (Tommasino *et al.*, 2012). According to Stark (2005), MDA content contributes to the loss of cellular activities by inactivating membrane enzymes and even cytoplasmic proteins through the formation of ROS. The data obtained from this experiment was found in accordance with the findings of Jincy *et al.* (2019). MDA content produced under ambient+6°C conditions was found to be significantly higher compared to remaining conditions.

Reactive oxygen species like superoxide radicals could induce membrane lipid peroxidation which leads to loss of membrane stability and produce MDA as a byproduct and this may be the reason for higher MDA content observed in UMI 1230 inbred line exposed to high temperature stresses such as ambient temperature + 4°C and 6°C. However, among two inbred lines, CBM-DL-322 recorded reduced MDA content compared to the UMI 1230 in both elevated temperature treatments which indicates that at the cellular level CBM-DL-322 maize inbred line is better managed with an efficient free radical quenching system that offers protection against oxidative stress due to high temperatures (Yadav *et al.*, 2018).

### Effect of High Temperature on Antioxidant Enzyme Activity

Plants use complex antioxidant defense systems to combat uncontrolled ROS production and protect themselves from oxidative damage. Under stressful conditions, all plant species require a balance between ROS production and antioxidant enzyme activity (Tesfaye *et al.*, 2018). Enzymatic antioxidants protect the plant cells from the oxidative damage caused by abiotic stresses through detoxification of ROS.

Against reactive oxygen species, plants have an enzymatic antioxidant defense system. SOD is the first line of defense in

such antioxidant system, catalysing the dismutation of  $O_2^-$  to  $H_2O_2$  and  $O_2$ . Gill and Tuteja (2010) reported that when plants were subjected to high temperature stress, the equilibrium between the production and scavenging of reactive oxygen species was disrupted, causing oxidative damage to cell membranes. Singh *et al.* (2020) reported that the activity of SOD in maize was significantly increased under elevated temperature conditions. In the present study also it was observed that under ambient temperature +4°C condition, SOD activity was found to be significantly increased in both UMI 1230 and CBM-DL-322 maize inbred lines. Due to the effective activation of the antioxidant machinery, the plants under oxidative stress could manage the stress effectively and avoid cell membrane damage and protein degradation. These findings were in accordance with Khayatnezhad and Gholamin (2021) that the synthesis of SOD is part of the plant's defensive mechanism against oxidative stress in moderately and highly tolerant plants under abiotic stress conditions.

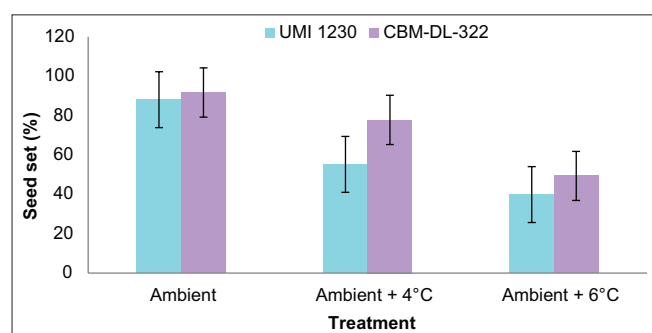
The ascorbate-glutathione cycle is the main hydrogen peroxide detoxification system in plant chloroplasts. Ascorbate peroxidase uses ascorbic acid as a specific electron donor to reduce  $H_2O_2$  to water. Caverzan *et al.* (2012) reported that the role of APX is not only limited to the chloroplast, it also occurs in the cytosol, mitochondria and peroxisome. Under high temperature conditions, ascorbic acid content was increased when compared to non-stressed plants (Xiang *et al.*, 2019). Similar result was also observed in this study in maize inbred lines under elevated temperature conditions. The APX enzyme is involved in hydrogen peroxide scavenging and its activity has been correlated with the relative tolerance of the crop (Sairam *et al.*, 1997). In current study, both maize inbred lines UMI 1230 and CBM-DL-322 recorded higher APX enzyme activity compared to ambient condition but among the inbreds, CBM-DL-322 registered the highest activity which indicates its relative ability to tolerate the temperature induced oxidative stress compared to UMI 1230. The increased activity of APX enzyme may be due to nonspecific plant defense responses under temperature stress. Zhu *et al.*, (2010) and Yuzbasioglu *et al.* (2017) reported that the ascorbate peroxidase activity was increased in maize seedlings when the temperature was increased from the ambient condition.

Catalase protects the cell from oxidative damage caused by reactive oxygen species by catalyzing the breakdown of  $H_2O_2$  into water and oxygen. In rice, Zafar *et al.* (2020) reported that catalase activity was doubled under elevated temperature stress. In the present study, also catalase activity increased under elevated temperature conditions in both the inbreds - UMI 1230 and CBM-DL-322 - compared to the ambient condition. Specifically, the maize inbred CBM-DL-322 registered a significant increase in CAT activity over the inbred CBM-DL-322 which indicates its potential defense against oxidative damage.

### Effect of High Temperature Stress on Yield Parameters

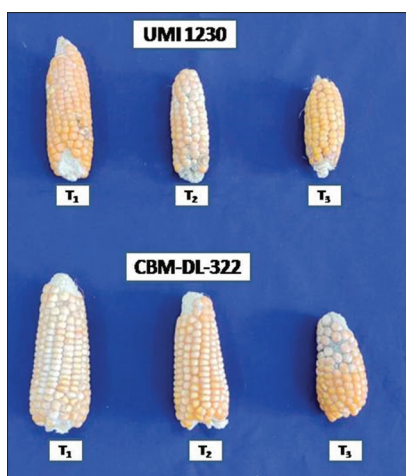
#### Cob weight

Regarding the cob weight, the findings were in agreement with the results of Shim *et al.*, (2017) who reported that the weight of cob



**Figure 4:** Effect of elevated temperature stress on seed set percentage in maize inbreds





**Figure 5:** Cob/ear length of maize inbreds subjected to elevated temperature condition. (Treatments: T<sub>1</sub>- ambient (control), T<sub>2</sub>-ambient+4°C, T<sub>3</sub>-ambient+6°C)

gets decreased in maize plants exposed to elevated temperatures during the reproductive stage. It may be due to increased lipid peroxidation (accumulation of MDA) which leads to the damage of cell membrane preceded by a reduction in photosynthetic rate and yield and quality of the grains in maize (Suwa *et al.*, 2010). However, increased accumulation of antioxidants in some of the genotypes resulting in a reduced level of membrane damage and further maintenance of normal photosynthesis under heat stress leads to increase in cob weight (Hasanuzzaman *et al.*, 2013). This is in accordance with the results of the present study in which CBM-DL-322 performed better at elevated temperature of plus 4°C among the two maize inbred lines.

#### Seed set percentage

From the results, it was observed that the seed setting percentage was reduced significantly by 15 and 54% when plants were exposed to the elevated temperatures of about 41 and 44°C, respectively during the reproductive stage (Figure 5). The reduction in seed set percentage was the consequence of disruption in source–sink relations by reducing the source capacity and sink formation as a feedback mechanism under elevated temperature stress conditions (Lamichaney *et al.*, 2021). Devasirvatham *et al.*, (2012) reported in chickpea that reduction in the pollen formation and its viability causes lower seed setting under high-temperature. However, among two inbred lines, CBM-DL-322 recorded a lesser reduction in seed set percentage compared to the UMI 1230 in both the elevated temperature treatments which indicates the ability of the inbred CBM-DL-322 to perform better under such high temperature conditions due to its inherent capacity to activate the antioxidant system to nullify the effects of reactive oxygen species and by the way better performance in terms of photosynthesis, partitioning of assimilates and yield.

#### CONCLUSION

During heat stress, ROS are produced in plants and cause membrane oxidation resulting in organelle degradation and cell

death. The present study revealed that the elevated temperatures beyond optimum showed a negative impact on cob weight and seed set percentage. This is due to the changes in membrane orientation in cell organelles such as chloroplast etc., which lead to a reduction in source capacity and sink formation and also pollen and its viability under elevated temperature condition. Further, it was observed a significant enhancement in antioxidant enzymes activities in the maize inbred CBM-DL-322 probably detoxified the ROS effectively compared to the UMI 1230 and hence, CBM-DL-322 managed to withstand under elevated temperatures and sustained the yield.

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