Impact of drought stress on morphological and yield components in maize (Zea mays L.)
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Abstract
Maize (Zea mays L.) is a major grain crop cultivated around the world. It is useful as a bio fuel as well as for human and animal nutrition. Increased abiotic and biotic stress events have been documented in many regions of the world as a direct result of global climate change, posing a threat to worldwide maize harvests. One of the most environmental factors is drought that affects maize crop growth, development and yield. Plants have evolved dynamic physiological, biochemical and molecular reactions that enable them to escape, avoid and survive harmful environmental conditions.

Drought, more than any other abiotic stress, is a significant cause of decreasing crop yields. Maize plants, as one of the most widely distributed crops, are regularly subjected to drought stress, resulting in significant losses in final kernel yield. Plants drought stressful events included tissue and developmental stage specific characteristics. These data suggest that induced stress reduced yield by reducing plant development and yield parameters as compared to control plants. As a result, these biochemical characteristics and physiological responses may be important in the development of drought tolerance genotypes that can withstand water deficits while retaining a high yield.

Keywords: Antioxidant, Drought stress, Yield characters, Reactive oxygen species, Zea mays.

Introduction
Maize is one of the world's most frequently farmed grains with significant grain yield and nutritional value 16. Crops are typically faced with numerous abiotic stresses simultaneously during their life cycle in the natural environment, significantly affecting field crop development and yield 65. Drought is the most prominent abiotic stress on crops and as a result, food security in a changing climate; these stresses significantly impact on the yield of important staple food crops which account for 60% of the global food energy supply. Seedling growth (VE and V1), vegetative growth (V2, V3... Vn), flowering and reproduction (VT, R0 and R1) and grain filling and maturity (R2 to R6) are the stages of maize growth 1.

During the vegetative period, this can result in a reduction in growth rate, extend the stage of vegetative growth and re-route the carbohydrate distribution in maize. During the rapid vegetative phase, 28–32 percent of dry weight is lost. During the development stage, there are 66–93% dry weight losses 16. Many potential ear shoots emerge during the V9 stage and the number of kernel rows is determined 41.

The maize plant begins with a rapid, significant rise in nutritional and dry weight accumulation around the V10 stage, which continues into the reproductive stage. Long-term drought (21 days) during the pre-flowering stages has also been demonstrated to decrease the terminal sizes of some leaves and internodes, delay tassel and silk emergence and results in yield losses of 15 to 25%. Drought stress at this stage will significantly lower the number of kernels per plant 9. Hence the potential of kernels per row is decided by at least V15 and possibly as early as V12 stage; drought stress for ten days around pollination induces abnormal embryo development and a significant decrease in kernel number. During the pre-pollination and early post-pollination stages, a reduced kernel set, mainly in the apical ear areas, has also been observed in response to 5 days of drought stress 99.

The photosynthesis of the maize ear leaf contributes significantly to the growth of biomass. Five or six leaves near and above the ear produce the majority of the photosynthate for kernel production, during the vegetative stage of maize. Drought stress reduces the active photosynthetic leaf area of the crop canopy, resulting in a loss of production at maturity as the anthesis-silking interval (ASI) is prolonged and grain weight is limited 57. On the other hand, drought stresses are more responsive to reproductive than vegetative stages of crop plants; however, each stress impacts reproductive traits differently 44.

Turgor loss which slows growth rate, stem elongation, foliar expansion and stomatal opening, changes the sink-source relationship, affects photosynthetic transfer to fruits and is the first response to stress 12. Antioxidant enzymes in cellular organelles and the cytoplasm such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, play a pivotal role in the detoxification of these reactive species 7. Osmotic adjustment is one of the most effective ways to protect plant cells from abiotic stress damage and it is associated with higher grain yield during drought 13. Glycine betaine, amino acids, proline and sugar solutes can accumulate and play an essential role in cell signaling turgor and protecting membranes and proteins from severe damage caused by water loss 21. During the reproductive phases of plants, osmolytes improve carbohydrate and soluble sugar partitioning, resulting in higher final yields 53. Proline is...
involved in quenching free radicals, maintaining sub-cellular structures and buffering cellular redox potential, among other things and accumulates in response to stress\(^8\).

Lipid peroxidation indicates the occurrence of radical reactions in tissues. Drought stress is known to cause an increase in lipid peroxidation in plants\(^38\). To cope with oxidative damage produced by drought stress, plants adapt their defensive mechanism, enzymatic or non-enzymatic\(^28\). Plants have an effective antioxidant (enzymatic and non-enzymatic) defence system against ROS-induced oxidative damage.

Under stressful conditions, enzyme antioxidants such as APX, SOD, CAT and POD prevent oxidative damage. Plants subjected to long-term drought stress may benefit from antioxidants that are both enzymatic and non-enzymatic\(^51\). These antioxidants have been shown to contribute directly or indirectly to maize drought tolerance; for example, prolonged yields in drought-stressed maize were directly connected to enhanced antioxidant activities, promoting drought tolerance by scavenging ROS\(^52\).

In this study, we investigated drought-induced oxidative damage in terms of ROS accumulation as well as potential protections such as osmoregulation and enzymatic defence system activation. The importance of these physio-biochemical systems in maize growth and yield response under drought stress was also investigated to acquire a better knowledge of maize tolerance to drought stress.

**Material and Methods**

**Plant Material, Growth Conditions and Experimental Design:** The maize (*Zea mays L.*) variety Co-6 was provided by TNAU, Tamil Nadu Agricultural University, Coimbatore. The seeds were surface-sterilized in 0.1g mercuric chloride for 10 minutes before being rinsed in distilled water before sowing. Ten seeds were planted in the field (in a 1:1:1 combination of red soil, sand and farmyard manure) and irrigated with tap water. The maize plants were let to grow in their natural environment until tasselled. During the reproductive stage, from the 56\(^{th}\) to the 75\(^{th}\) day, drought treatment withholding water was supplied for 20 days. The plants were harvested on the 120\(^{th}\) day. The experimental treatments were arranged in a completely randomized design under the factorial arrangement (CRD).

**Measurement of growth, biomass and yield parameters:** Plant height, tassel length, corn height and corn diameter were measured using a meter scale while the electronic weighing balance was used to assess the shoot fresh and dry weights. Relative water content (RWC) of maize plants was assessed following the method of Chen et al\(^19\):

\[
RWC (%) = \frac{(FW-DW)}{FW} \times 100
\]

where FW indicates fresh weight, DW represents the dry weight and 100 is the factor used to determine RWC in maize, all characters were measured on the 76\(^{th}\) day. For the analysis of yield parameters on the 120\(^{th}\) day, three plants from each replication were randomly sampled and harvested at maturity. After sun-drying, the ears were manually shelled and yield constituents were analyzed.

**Photosynthetic components and water relations (mg g\(^{-1}\) FW):** At 20 days after drought stress treatments, photosynthetic components were measured on intact leaves from the fourth branch from the top. 250 mg leaf without vein (leaf blade) was extracted and the extract was transferred to a 15 ml tube. For 24 hours, the tubes were kept in the dark to avoid exposure to light. The absorbance was measured at 663 nm, 645 nm and 480 nm for chl a, b and carotenoid and expressed in mg g\(^{-1}\) FW.

**Biochemical Parameters:** Five hundred milligrams of fresh shoot and root were mashed in a centrifuge with 10 ml of 80 percent ethanol and the homogenate was spun at 8000 rpm for 10 minutes. The particle was re-extracted with boiling 80 percent ethanol from the supernatant and the supernatants were pooled together. The ethanol in the supernatant was evaporated and the aliquot was made up to 20 ml with distilled water. The sugar content of the collected liquid phase was measured. All reagents containing extract were used as blanks\(^40\).

In a pestle and mortar, grind 500 mg of fresh plant tissue with 10 ml of 20% trichloroacetic acid (TCA). The homogenate was spun at 8000 rpm for 20 minutes in a cooling centrifuge. The mobile phase was discarded and the pellet was suspended in 1 ml of 0.1 N sodium hydroxides (NaOH) to solubilize the protein which was estimated by Bates et al\(^11\).

In a pestle and mortar, macerate 500 mg of fresh frozen plant material with 10 ml of 3 percent aqueous sulphosalicylic acid. Whatmann no. 1 filter paper was used to filter the homogenate. The residue was re-extracted and pooled and an aliquot was prepared up to 20 ml with aqueous sulphosalicylic acid, which was used to determine proline content\(^35\). 500 mg of plant tissue were homogenized with 5 ml of 80 percent boiling ethanol. The homogenized material was centrifuged for 15 minutes at 8000 rpm and the supernatant was diluted with 80 percent ethanol to make up to 10 ml. The absorbance was measured and quantified as mg g\(^{-1}\) FW sample solution\(^37\).

**Lipid peroxidation rate and ROS accumulation (µ mol g\(^{-1}\) FW):** Malondialdehyde (MDA) content determines the level of lipid peroxidation\(^37\). 2 ml aliquot of enzyme solution was added to a tube containing 1 ml trichloroacetic acid (20% v/v) and 0.5 percent thiobarbituric acid (0.5%). The combination was heated to 95-9°C for 30 minutes, cooled to room temperature and then spun for 10 minutes at 14,000 rpm. The absorbance of the supernatant at 532 nm was measured and a nonspecific absorbance at 600 nm was subtracted.
Antioxidant activities (Unit mg\(^{-1}\) protein): 500 mg of frozen plant material was crushed in 5 ml of ice-cold 50 mM phosphate buffer (pH 7.0). The homogenate was spun at 5500 rpm for 20 minutes in a cooling centrifuge at 4°C. The supernatant is employed in antioxidant enzyme assays such as catalase\(^{18}\), peroxidase\(^{17}\).

Results and Discussion
Drought stress is a crucial impediment to increased field crop growth and yield. The present study looked at how drought stress affected maize growth, yield and other characteristics. Previously, drought stress during pre-tasseling and high temperatures around the anthesis stage reduced maize growth and considerable production losses. Furthermore, substantial decreases in growth, osmolyte accumulation, antioxidant defense system and ROS-based changes in maize growth and yield performance were observed under drought stress at various reproductive stages. Several studies have previously documented the adverse effects of combined drought and heat shocks on the development and yields of several cereal crops; however, the level of damage under these pressures varies with the intensity of stress and crop growth stage.

Plant height was retarded under drought stress conditions in maize. During the recovery stage, they increased their growth rate but the seedling was significantly shorter than the length of the controls. A considerable decrease in growth in drought-exposed maize cultivars resulted in lower fresh and dry biomass than in respective controls. Fresh biomass of cultivars was reduced under drought stress conditions, but it was increased during control. In addition, dry biomass was significantly decreased under drought stress compared to their controls. RWC was significantly increased under drought stress conditions, but it decreased during control leaves (Figure 1).

Low turgor pressure severely restricts cell growth and proliferation in drought-stressed plants, although osmotic regulation may prevent this by preserving cell turgor\(^{50}\). Drought stress reduces leaf area, which reduces photosynthesis and crop yield; expansions depend on leaf turgor temperature and assimilate availability, both of which are impacted by drought\(^{46}\). Under drought conditions, the relative water content of sunflower leaves increased. A reduction in leaf RWC indicates a decrease in swelling pressure in plant cells which causes growth to slow\(^{33}\).

![Figure 1: Effect of drought on maize plants at 76th day](image-url)
- a) Plant height
- b) Fresh weight shoot, Fresh weight root
- c) Dry weight shoot, Dry weight root
- d) Relative water content shoot, Relative water content root
- e) Photosynthetic pigments
- f) Reducing sugar shoot, Reducing sugar root
Drought stress is shown to impair maize plant growth and development including leaf area, the number of leaves and leaf length, with a decrease in plant fresh and dry biomass, which is one of the primary drought stress indicators\textsuperscript{14}. Similar results were seen in chickpea when drought stress was introduced at pre-tassel and high temperatures at anthesis and reproductive phases\textsuperscript{25}. The chlorophyll content (\textit{a, b}) of maize cultivars was significantly reduced under drought stress, but it increased and reached the control values during recovery (Figure 1). The substantial drought-induced decrease of the chl content indicates that the drought stress induced an intense loss of photosynthetic reaction (PSI and PSII).

At the same time, the carotenoid content was increased during the drought treatment leaves compared to control leaves\textsuperscript{23}. Plants use photosynthetic pigments primarily for light absorption and the synthesis of reducing powers. The chl \textit{a} and \textit{b} are both susceptible to soil drought; on the other hand, carotenoids play additional roles in the plants ability to withstand drought.

However, drought primarily inhibits photosynthesis through stomatal closure, or metabolic damage continues to be debated \textsuperscript{31}. Although both stomatal and non-stomatal limitations were widely accepted as the main factors of reduced photosynthesis under drought stress, photosynthesis under drought due to metabolic impairment is a more complex phenomenon than stomatal limitation. It is primarily caused by reduced photosynthetic pigment contents in sunflowers under drought, the contents of photosynthetic pigments chl \textit{a} and chl \textit{b} were reduced while carotenoids increased, compared to the control \textsuperscript{46}.

Chlorophyll is an essential component of plants that helps them grow and develop through photosynthesis \textsuperscript{43}. Photosynthetic pigments absorb sunlight and use this energy to produce glucose, which is then disseminated through a separate biosynthetic route. Drought has been shown to cause a decrease in these pigments, putting the plant prone to death\textsuperscript{10}. According to a study on potatoes, the stomatal restriction factor, photosystem II damage and the antioxidant enzyme system are all responsible for decreasing photosynthesis rate in drought-susceptible plants reducing the chlorophyll \textit{a, b} content in maize leaves compared to control\textsuperscript{32}.

Biochemical parameters such as reducing sugar and protein were decreased in drought compared to the control. In contrast, proline and amino acid were increased in drought leaves compared to the control. Different osmolytes were produced and accumulated in maize due to drought stress. Drought conditions resulted in much higher reducing sugar, protein, proline and amino acid concentrations than the well-watered control (Figure 1, 2). Drought stress caused the lowering sugar control plants to produce more sugar. Sugars are the principal substrates of respiratory metabolism; the rate of respiration is directly connected to the amount of sugar in plant tissue and soluble sugars play a crucial role in plant structure and metabolism at both the cellular and whole-organism levels\textsuperscript{32}.

Sugar accumulation increased significantly in rice drought leaves compared to control plants, indicating that sugar accumulation positively regulates the osmotic balance and serves as a drought stress protection mechanism\textsuperscript{33}.

Our results showed that sugars slightly influenced osmotic adjustment and that water loss may have caused osmotic alterations as a side effect. Osmotic potential is a good indicator of plant water status and soluble sugar build-up in maize plants under stress may play a role in ROS scavenging and signaling pathways. Drought stress led to an increase in soluble proteins, proline and amino acids compared to control plants. Protein biosynthesis, cell defense, signal transmission, transport and lignifications have been demonstrated to play a vital part in plants’ adaptive response to drought in the study of drought-sensitive proteins\textsuperscript{47}.

On the other hand, peanut drought leaves showed a marked difference from their wet counterparts\textsuperscript{48}. Proline content was more significant in maize leaves grown under extreme water stress than in unstressed control plants\textsuperscript{1}. Proline levels in \textit{Salicornia brachiata} rose significantly when water stress was generated by PEG, indicating that it played a dynamic role in osmotic regulation and free radical scavenging to offset the stress\textsuperscript{42}. \textit{Salicornia brachiata}, a plant that plays an essential role in osmotic control, cellular defense and free radical stone during drought stress, had an elevated build-up of free amino acids when grown in drought\textsuperscript{24}. Under drought circumstances, respiration rate increased with an increase in the concentration of amino acids in the root system of wheat.

MDA values in maize leaves show that drought and control plants had varying degrees of lipid peroxidation\textsuperscript{29}. There was an increase in MDA content in the drought-stricken group compared to a control group (Figure 2). Cold-treated wheat plants also showed greater MDA levels than control plants, supporting the well-known impact of cold stress on membrane integrity\textsuperscript{38}. Similar studies in wheat leaves show that drought stress raises MDA levels, which affects the activity of intercellular antioxidant enzymes and enhances the plant's ability to withstand environmental stressors\textsuperscript{35}.

Drought stress enhanced lipid peroxidation and protein oxidation in peanut leaves, but these values reverted to their pre-drought levels when the leaves were rehydrated. A rise in H$_2$O$_2$ indicates an increase in oxidative stress and ROS generation, showing oxidative stress in this case. Stressed peanut nodules were shown to have lipid but not protein damage, which decreased during rehydration. Similarly, legume nodules subjected to drought in high ROS level\textsuperscript{39} reported similar outcomes.

Plant cells react to oxidative stress with caution, scavenging ROS and retaining antioxidant defense molecules at levels...
that reflect ambient environmental circumstances. The stress-induced build up of ROS may be prevented by modulating antioxidants under abnormal circumstances. Drought-stressed plants had higher levels of CAT and POD than those in the control group.

Enzymatic antioxidants such as catalase and peroxidase were increased in drought plants compared to the control (Figure 2). It was shown that catalase activity was enhanced in all rice genotypes investigated compared to the control when water stress was imposed. CAT activity increased considerably during the blooming and milking periods with the maximum activity occurring during the flowering stage under acute water stress. Catalase activity was found to be higher in lettuce plants subjected to drought stress than in control plants. In addition, the severity of the drought has a considerable impact on the amount of activity of the CAT.

Drought-stressed plants such as sunflowers show increased POD activity compared to their control counterparts. The decreased O₂, H₂O₂ and MDA levels in maize plants may be due to increased POD and CAT activity which may efficiently scavenge ROS. Osmotic stress increases POD activity in both drought-resistant and susceptible maize plants.

Figure 2: Effect of drought on maize plants at 76th day a) Amino acid b) Protein c) Proline d) MDA e) Catalase f) Peroxidase
Significant differences in cob mass were seen between control crops and drought crops. The maximum cob length was measured in control and it was much lower than the minimum cob length drought in this experiment. Drought-induced stress reduced the girth and length of cobs, although the 100-grain weight of the cobs was much more significant in control than in the drought condition. The stress weight was 100 grains, much less than the control weight (Figure 3, 4).

Figure 3: Effect of drought on maize plants at 120th day a) Tassel length b) Corn height c) Corn breadth d) 100 seed weight

Figure 4: Effect of drought on maize plants at 120th day a) Plant length b) Corn height c) Seed Size
When drought stress was administered to maize during the blooming phase, it delayed silking and extended anthesis silking interval (ASI) in late stress which resulted in reduced grain yields. Grain weight, kernels per cob, grain output per plant and harvest index were all significantly reduced by drought stress on maize plants during the tasseling stage of growth. Wheat growth, development and yield may depend on the vegetative stage under dry circumstances. Drought stress dramatically decreased grain yield and yield components in maize, primarily due to a considerable drop in the number of kernels per cob. Similar findings were found for grain yield and drought adaptation in maize.

**Conclusion**
Maize yield and growth were connected to ROS generation, osmolyte build-up and activation of antioxidant defence systems under drought circumstances. Identifying drought-resistant cultivars and improving the resilience of maize to drought stress might benefit from some of these aspects. Even if water is accessible in the field, new plant growth regulators may be developed based on suitable morphological, physiological and biochemical characteristics. A better knowledge of the causes of poor yield and quality may lead to higher drought tolerance via both old and new strategies.

Further investigation is necessary to find the mechanism of seed priming and the foliar application of growth regulators in drought-stressed maize. Using these methods in conjunction with genetic modifications may also be considered in more integrated ways. The potential use of foliar abscisic acid to produce maize hybrids that can mitigate the negative effects of drought and heat stress on maize plant growth and development is being investigated.

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