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Evaluation of Oxidant and Antioxidant Systems in Drought Response of Cowpea Varieties

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Abstract
Plant in their natural environment are exposed to several stresses such as the drought that affect their growth and productivity. Exposure of plant to drought leads to oxidative stresses as a result of increase in ROS, however plant counteract this effect by its antioxidant system. H_2O_2, malondialdehyde, membrane stability index, catalase and ascorbate peroxidase activities where evaluated in leaves of two cowpea varieties drought tolerant (Dan'ila) and drought susceptible (TVU 7778) during 2 weeks water deficit and 5days recovery. The result indicates significant increase in the H_2O_2, MDA, CAT and APX activities in both varieties during the stress period and significant decrease during recovery in drought tolerant variety compared to the drought susceptible variety. The membrane stability index decreases significantly during stress period in both varieties. Drought tolerant variety has the higher ability to counteract oxidant activity during the drought stress and recover faster than the drought susceptible variety.
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1. Introduction

Plants are exposed to different kinds of stress which may originate from human or natural causes such as drought. Drought stress severely impacts plant development, growth and fertility. Stress imposed stress on plants commonly accompanied by an increase in the production of reactive oxygen species (ROS) that lead to an imbalance between their production and scavenging. Despite their reactive and thus toxic nature, ROS are also key components of signal transduction pathways that trigger stress responses. Furthermore, ROS are involved in plant developmental processes (Mhamdi and Breusegem, 2018) and plant-microbe interactions (Segal and Wilson, 2018). However, excessive ROS production must be counteracted by the antioxidant system to prevent damage development and cell death. The first response of plants to drought is the closure of stomata in order to minimize water loss due to transpiration. Depletion of the intercellular carbon dioxide (CO_2) concentration stimulates ribulose–1,5–bisphosphate oxygenation and, thus, photorespiratory hydrogen peroxide (H_2O_2) production in the peroxisomes by the dismutation of $O_2\bullet^-$ .Sequence of detoxification steps is often required to avoid the conversion of one reactives pecie to 2nd more harmful one such as the OH radical Detoxification of H_2O_2 to and o_2 is achieved primarily by the action of catalase in the peroxisomes and ascorbate peroxides in the chloroplast. Drought also induces lipid peroxidation leading to disruption of cell membrane.

1.1.0 Materials and methods

The experiment was conducted between September to October 2018 at a greenhouse of Faculty of Agriculture, Bayero university, Kano (approximate temperature of 35/28˚C (day/night), 50–80% of relative humidity and 13 h photoperiod recorded with data log (11° 58´ N and 8° 25´ E 457m above sea level) in the savanna ecological zone of Nigeria. Two beans variety drought tolerant Dan-ila and Drought susceptible TVU 7778 were collected from International Institute of Tropical agriculture. Before sowing, seeds were surface sterilized with 2.5% sodium hypochlorite for 20 min, then were washed with sterile deionized water. The experimental pots 12cm×8cm 5 liter capacity were used and arranged in a simple randomized design with six replicates per treatment per variety. Five seeds were sown per pot at the depth of 3cm which were later thinned to 3 seedling per pot at 2 weeks after sowing. Spacing of 20cm inter row and 10cm intra row was used in the pots arrangement. Each pot was irrigated to field capacity once daily with borehole water having 7.5 pH, 1.2 dsm^{-1} electrical conductivity (EC) and 1200 mg L^{-1} total soluble salts (TSS) until the start of the stress treatments. Water deficit was introduced at 5weeks after sowing by totally withholding irrigation for a period of 2 weeks for the stressed group. Irrigation was resumed for 5 days to assess level of recovery. Sample of the plant leaves were collected at

0, 5, 10, 15 days drought and 5 days after re-watering for biochemical analyses.

Fully expanded leaves were collected in the morning and then washed with deionized water; the adhering water was removed with a paper towel. The leaf samples were immediately frozen in liquid nitrogen to prevent proteolytic activity and taken to the Laboratory for analysis.

1.1.1 Antioxidant enzymes Extraction

Enzyme extract for catalase (CAT) was prepared by weighing (1 g) followed by grinding with 5 ml of cold extraction buffer (0.1 M phosphate buffer, pH 7.8, containing 0.5 mM EDTA, and 2% (w/v) polyvinylpyrrolidone (PVP), filtrate was centrifuged for 20 min at 10,000 g and the supernatant was used as enzyme extract. All steps in the preparation of the enzyme extract were carried out at 4°C. For measuring ascorbate peroxidase (APX) activity, the leaves was separately grounded in homogenizing medium containing 2.0 mM AsA in addition to the other previous ingredients.

1.1.2 Ascorbate peroxidase activity

Ascorbate Peroxidase (APX) activity was assayed following methods adopted by Nakano and Asada (1981). The reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.5 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was started with the addition of H_2O_2. Absorbance change was measured at 290 nm every 30 s for 5 min (ε = 2.8 mM cm^{-1}) using spectrophotometer. APX activity was expressed as Units per gram fresh.

1.1.3 Catalase activity

Catalase activity (CAT) was determined following the reaction of the extract in the presence of 50 mM potassium phosphate buffer (pH 7.0) containing 12.5 mm H_2O_2 and 50 µl enzyme extract and water was made up to 3.0 ml. The reaction took place at 25 °C, by adding H_2O_2 with absorbance monitored at 240 nm for 60 s (Aebi, 1984). CAT specific activity was expressed as Units per gram fresh weight calculated using the molar absorptivity of 43.6 mM^{-1} cm^{-1} for H_2O_2 at 240 nm.

1.1.4 Hydrogen peroxide

To determine the level of hydrogen peroxide, 0.3 g of leaf tissue was homogenized with 5 mL 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × g for 5 minutes. Then, 0.25 mL of the supernatant was mixed with 0.25 mL of 100 mM K-phosphate buffer and 0.5 mL of 1M KI. The absorbance was measured at 390 nm with a spectrophotometer. Hydrogen peroxide level was calculated using a standard curve prepared with known H_2O_2 concentrations (Alexieva *et al*. 2001).

1.1.5 Lipid peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) content in the leaves according to the method of Dhindsa *et al*. (1981). 5 mL of trichloroacetic acid (0.1% TCA) was added to 0.3 g of leaf tissues and homogenized completely. The homogenated samples were centrifuged at 10,000 × g for 5 min. at 4°C. The supernatant (0.3 ml) was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA, and incubated at 95°C for 30 min. The reaction was stopped by putting the sample in an ice bath for 5 min.; samples were centrifuged at 10,000 × g for 10 min. at 25°C. The absorbance of the supernatant was read at 532 nm using spectrophotometer. After deducting the non-specific absorbance at 600 nm, the extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the MDA concentration.

1.1.6 Membrane stability index and membrane injury

Membrane stability index and membrane injury. The membrane stability index (MSI) was measured by using a conductivity meter following the method of Khanna-Chopra and Selote, (2007). Leaf samples of 200 mg were thoroughly washed in double distilled water and placed in two separate 10 mL tubes of distilled water. One tube was heated for 30 min at 40 °C in a water bath and electrical conductivity was measured (C1). The second set was boiled for 10 min at 100 °C in a boiling water bath and electrical conductivity was measured (C2). The MSI was estimated by the equation given below:

$$MSI = \left[1 - \frac{(C1)}{C2}\right] * 100$$

Membrane injury (MI) was estimated as ratio of MSI of drought-stressed plants and MSI of control plants as given by Dhanda *et al*.2004

$$M\ I\ (\%) = \left[1 - \frac{(MSId)}{MSIC}\right] * 100$$

1.2 Statistical analysis

Comparison between groups were performed using Student's paired t-test. Data was expressed as mean ±Standard deviation. Significant difference was placed at $p < 0.05$.

1.3 Results

1.3.1 Changes in H_2O_2 and MDA contents.

Drought stress caused an increase in H_2O_2 and MDA concentrations (Figure 1and 2) and a decrease in

Membrane stability index (Figure 3) in drought susceptible variety (TVU 7778) compared to drought tolerant variety (Danila).However, the drought tolerant variety showed a faster recovery upon re-watering than the drought susceptible variety.

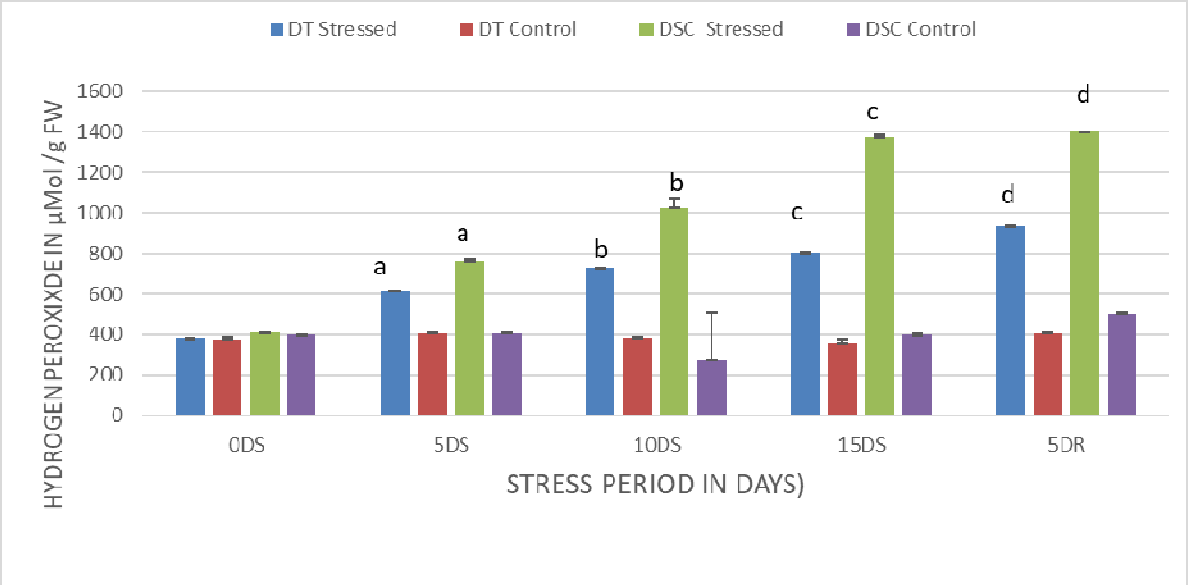

Figure 1: Effect of drought stress on hydrogen peroxide concentration in drought tolerant and drought susceptible cowpea varieties. Values within the same stress period bearing same alphabet are significant at P<0.05, DSC=drought susceptible, DT=drought tolerant, DS=days of stress, DR=days of rewatering.

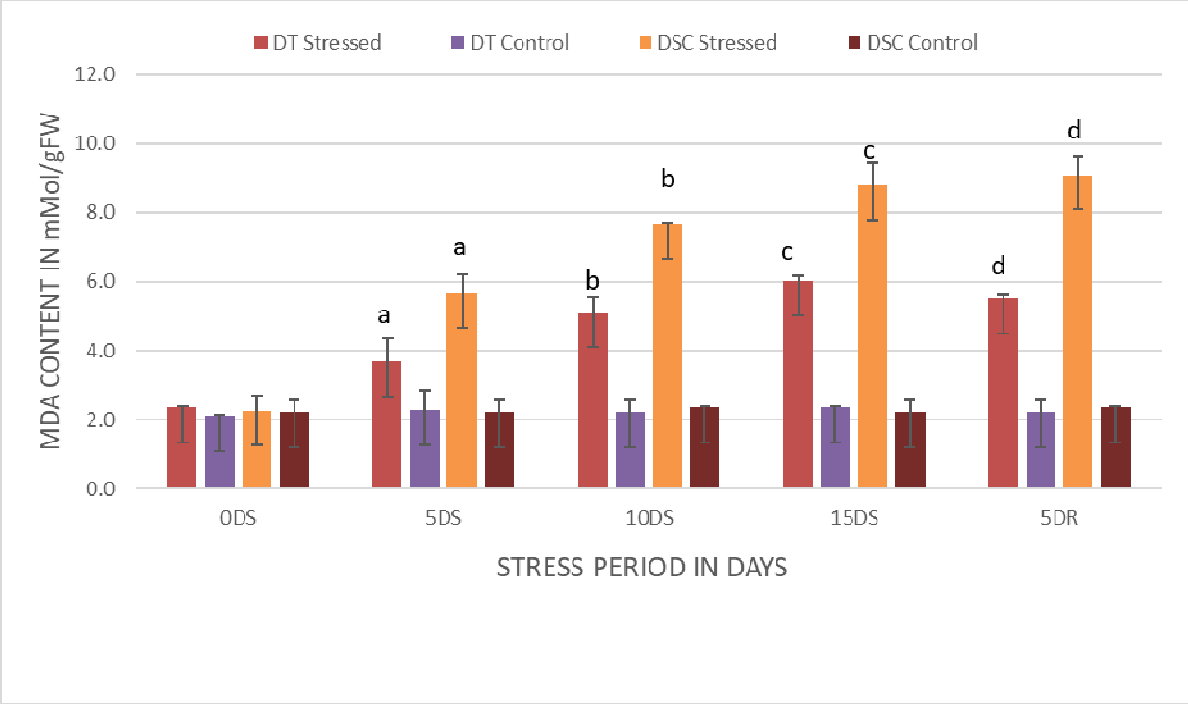

Figure 2: Effect of drought stress on malondialdehyde content in drought tolerant and drought susceptible cowpea varieties .Values within the same stress period bearing same alphabet are significant at p<0.05. MDA=malondialdehyde, DSC=drought susceptible, DT=drought tolerant, DS=days of stress, DR=days of rewatering.

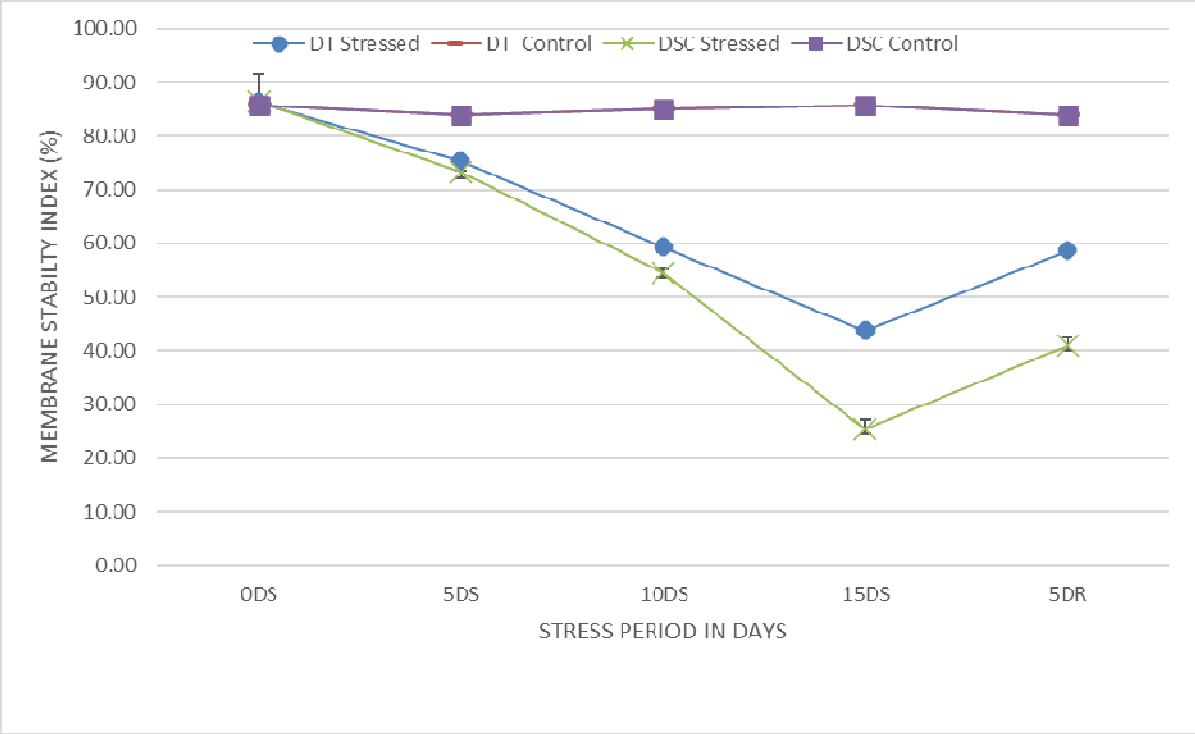

Figure 3: Effect of drought stress on membrane stability index in drought tolerant and drought susceptible cowpea varieties. DSC=drought susceptible, DT=drought tolerant, DS=days of stress, DR=days of rewatering.

1.3.2 Changes in enzymatic antioxidant activities.

During drought stress, the enzymatic activities of CAT and APX increased (Fig. 4 and 5). A rapid increase in the activities of CAT and APX was observed, which reached a maximum at 15DS .Overall antioxidant enzyme activities were higher in drought tolerant than drought susceptible with significant difference ($P \leq 0.05$) between the stress period. After re-watering, CAT and APX activities decreased significantly in drought tolerant plants as compared to drought susceptible plants. By comparison, in Drought tolerant plants, enzyme activities recovered to the level of control plants on 10DS whereas in drought susceptible the decrease is insignificant ($P>0.05$).

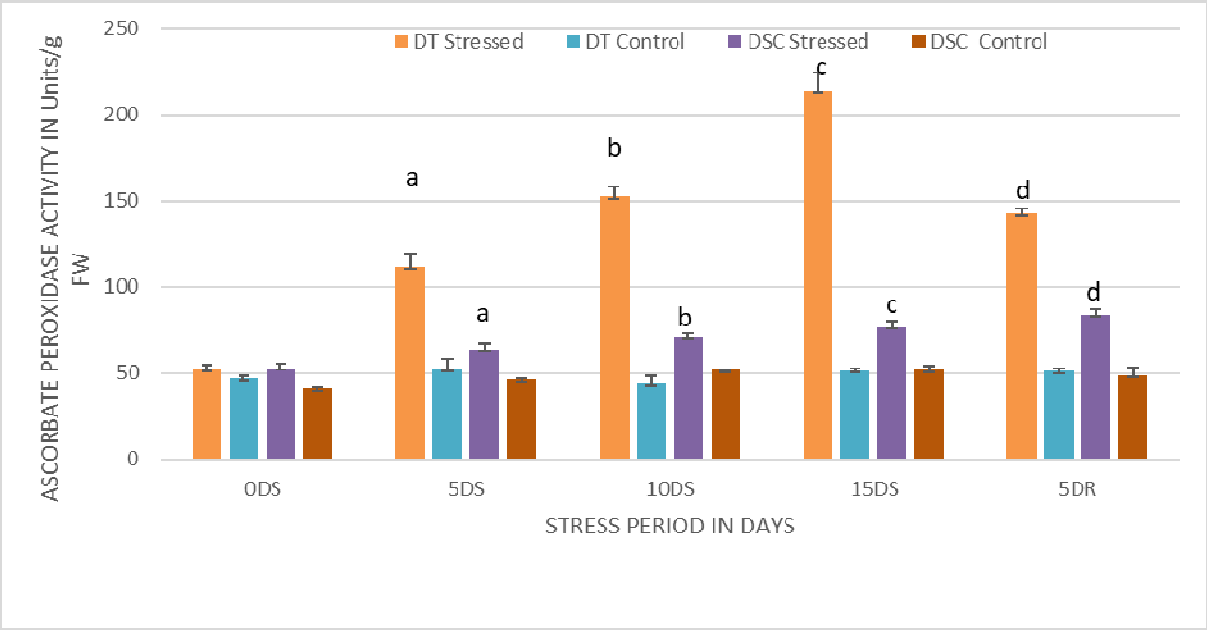

Figure 4: Effect of drought stress on ascorbate peroxidase activity in drought tolerant and drought susceptible cowpea varieties. Values within the same stress period bearing same alphabet are significant at $p<0.05$. DSC=drought susceptible, DT=drought tolerant, DS=days of stress, DR=days of rewatering.

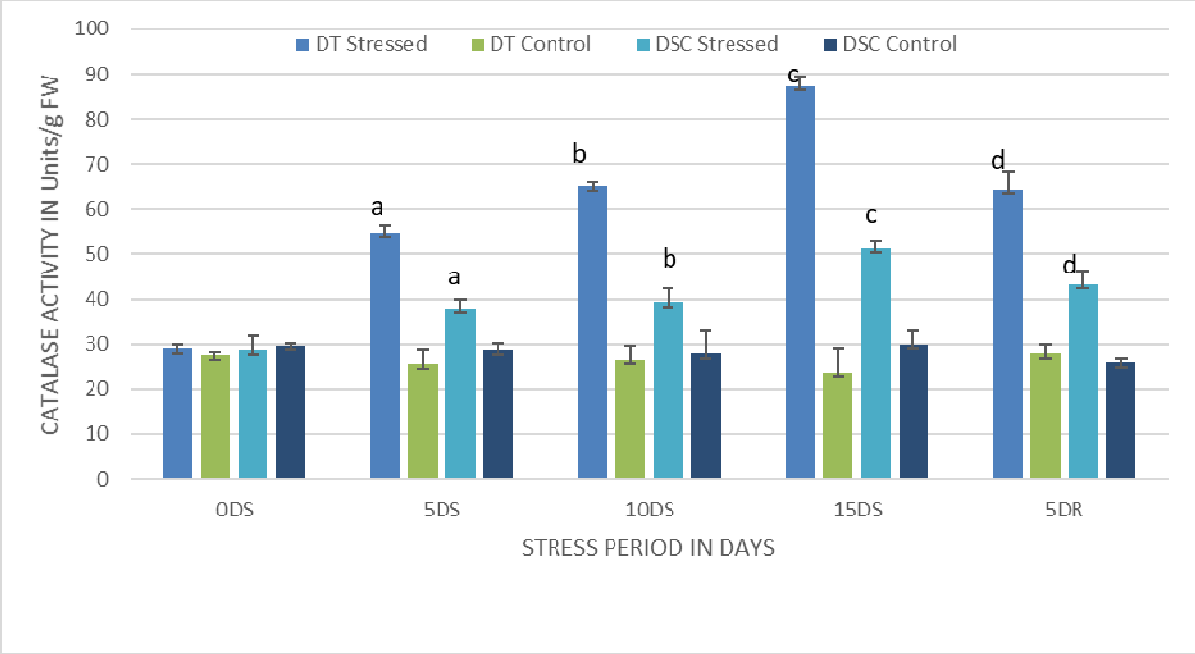

Figure 5: effect of drought stress on catalase activity in drought tolerant and drought susceptible cowpea varieties. Values within the same stress period bearing same alphabet are significant at $p<0.05$. DSC=drought susceptible, DT=drought tolerant, DS=days of stress, DR=days of rewatering.

1.4 Discussion

Reactive oxygen species such as hydrogen peroxide are constantly being generated at normal metabolic levels. However they are unable to cause damage as they are being scavenged by different antioxidant mechanisms (Foyer and Noctor, 2000).The balance between ROS generation and ROS scavenging is disturbed by different types of stress factors like salinity, drought and etc. Drought stress stimulates the production of ROS (Lui and Huang ,2000) because of stomatal aperture closure and low availability of CO_2 and its fixation Ribulose 1,5-bisphophate oxygenation is favoured and thus photorespiration is enhanced which account for more than 70% of the H_2O_2 produced as result of drought stress (Noctor *et al,* 2002).There was a significant increase in the amount of H_2O_2 ($P<0.05$) as drought stress is increased in the two varieties of cowpea but Drought tolerant specie showed a speedy recovery during rewarering than the drought susceptible, however the unstressed plants of both varieties showed similar index. Increased in lipid peroxidation shown by significant rise in MDA and reduction in membrane stability was also seen during drought stress period which is in line with the findings of (Zlatev and Lidon 2012) that says increased ROS cause membrane injuries that translate to decrease in membrane stability .The result thus indicates the degree of membrane damage was higher due to water stress (Chowdhury *et al* 2017.

Drought caused a distinct increase in the CAT and APX (Fig. 3 and 4) activities with Danila displaying some signalling molecules, such as oxidative molecules, may cause an increase in the antioxidant capacity of cell (Leila *et al* 2017). To alleviate cellular injury, stressed plants produce antioxidant enzymes (Zlatev and Lidon 2012). In leaf tissue CAT is localized in peroxisomes to scavenge H_2O_2 produced by glycolate oxidase in C2 photorespiration. The peroxisome is linked to the photosynthetic metabolism via photorespiration process and according to some findings may make part of oxidative tolerance (Luis and Eduardo, 2016). APX has an important role in the AsA-GHs cycle under stress activity of this enzyme is usually considered as an indicator of plant tolerance against the stress (Osman, 2015).The AsA-GSH cycle is known to be responsible for H_2O_2 scavenging in chloroplast (Asada,1992). As a consequence of CAT and APX's role in scavenging H_2O_2, an increase in CAT and APX activities can be regarded as a defence mechanism of the plant against the reinforcement of oxidative processes.

Conclusion

The cowpea plants subjected to drought had higher MDA, H_2O_2 and MSI in drought tolerant compared to the drought susceptible and control. CAT and APX activities involving enzymatic antioxidants were increased due to the stress caused by drought. Based on the results of this study, it is clear that water-deficit conditions affects the oxidant and antioxidant system of cowpea plant with the drought tolerant variety showing remarkable positive response than the drought susceptible suggesting that enzymatic antioxidant system plays a vital role in drought tolerant of cowpea plant.

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