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ORIGINAL ARTICLE

Antioxidative Response of Various Cultivars of Sorghum (*Sorghum bicolor* L.) to Drought Stress

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The present study was conducted with the aim to identify the response of antioxidant enzyme activities in seedlings of different sorghum cultivars under mannitol stress. Seven-day old seedlings were subjected to 100-500 mM mannitol stress which resulted in the decreases in shoot/root length and relative water content thus indicating the primary response to these tissues at phenotypic level. The level of lipid peroxidation as well as the specific activity of antioxidant enzymes such as peroxidase, catalase and superoxide dismutase increased at higher conc. except at 200 mM conditions. The level of catalase and peroxidase decreased at 500 mM conc. In the two different cultivars whereas the activity of superoxide dismutase consistently increased in response to the mannitol stress. Our data demonstrate that drought responsiveness tolerance in sorghum cultivars during germination is associated with enhanced activity of antioxidant enzymes.

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Key words: Catalase, drought stress, peroxidase, relative water content, superoxide dismutase, sorghum bicolor.

Abiotic stress conditions such as drought, heat, salinity, etc. are major threats to agriculture and lead to a series of morphological, physiological and molecular level changes that adversely affect the plant growth thus ultimately resulting in heavy loss of crop productivity (Boyer, 1982). Among the different environmental stresses drought is the most significant factor that restrict the plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas, 2007). It inhibits the photosynthesis of plants, causes causing the retardation of stem and root growth (Blum *et al*., 1997; Munns, 2002), a decrease in the assimilating leaf area (Passioura, 1988), changes in chlorophyll contents and components and damage to the photosynthetic apparatus (Nayyer and Gupta, 2006)

Drought stress lead to oxidative stress through the increase in reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2004, Apel and Hirt, 2004). To keep the levels of active oxygen species under control, plants generates non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage (Mittler, 2002) Non-enzymatic antioxidants including β-carotenes, ascorbic acid (AA), α-tocopherol (α-toc), reduced glutathione (GSH) and enzymes including, superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR)(Xu *et al*., 2008). The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants (Tsugene *et al*., 1999). Furthermore, the reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of developmeant (Chaves *et al*., 2003; Dacosta and Huang, 2007)

Sorghum (*Sorghum bicolor*) is the fifth most important cereal grown worldwide in terms of production. Like other millets, sorghum is genetically suited to hot and dry ecologies, where it is difficult to grow most of the other food grain crops. Sorghum, therefore, is an important crop for the food security in the semi-arid zones of Western Africa and India. In present study, effect of drought stress at different osmotic potentials on shoot length, total fresh and dry weight, relative water content and antioxidant enzymes in two sorghum cultivars were investigated.

MATERIALS AND METHODS

Seed of two cultivars of sorghum variety (GK-101 and GK-909) were germinated in 2 sets of six petridishes having moistened filter paper in a growth chamber at 25°C, 12 h light /12 h dark period, (illumination of 2500 Lux, Philips T2 40W/33 lamp) and irrigated daily with distilled water. Seeding was transferred to the 100 - 500 mM solution of mannitol (corresponding to -0.22 to -1.1 MPa osmotic stress) after six days growth. The samples were harvested and stored at -20°C for further analysis.

Relative water content (RWC) was estimated in the leaves for each drought period. Samples (0.5 g of the seedling tissue) were saturated in 100 ml distilled water for 24 h at 4°C in the dark and their turgid weights were recorded. The samples were oven-dried at 65°C for 48 h and their dry weights were recorded. RWC was calculated as follows, RWC (%) = [(FW – DW) / (TW – DW)] × 100, where FW, DW, and TW are fresh weight, dry weight and turgid weight, respectively.

Lipid peroxidation was measured in terms of content of malondialdehyde (MDA, ε = 155 $mmol^{-1}$ cm^{-1}), a product of lipid peroxidation, following the method of Heath and Packer (1968). 0.5 g seedling was homogenized in 10 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 5 min. To 1 ml aliquot of supernatant was mixed in 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was added and the mixture was heated at 95°C for 30 min and finally cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. MDA content was expressed as µmol g^{-1} DM.

CAT activity was assayed in a reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 30% (w/v) H_2O_2 and 0.5 mL of enzyme extract using the method described by Abei (1984). The reaction was started by the addition of enzyme extract. The activity of catalase was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H_2O_2 consumed. Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2 following the procedure described by Klapheck *et al*., (1990). The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm The reaction solution was 3 mL containing 10 mM (KH_2PO_4/K_2HPO_4) pH 7.0, 10 mM H_2O_2, 20 mM guaiacol and 0.5 mL enzyme extract.

SOD activity was determined according to the method described by Roth and Gilbert (1984). One millilitre of reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 100 mM EDTA, 20 ml enzyme extract and 10 mM pyrogallol. The enzyme activity [U (mg protein) 21] was calculated by monitoring the reaction mixture for 120 s (at 60 s intervals) at 420 nm on a Nanovue® device.

Statistical analysis

Mean and standard error (SD) values of three replicates were calculated. All data were diagnosed for normality of distribution and homogeneity of variance before being subjected to parametric statistical tests. Data have passed the normality and equal variance tests. All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences at $P \leq 0.05$.

RESULTS

Effect on the shoot/root length and relative water content

The effect of drought stress on the various physiological and biochemical aspects were studied on two sorghum cultivars. It was observed that there was a gradual decrease in shoot length both the cultivars with 40-58% decrease in GK 909 as compared to control sample (Fig. 1). In cv. GK 101 the decrease was less prominent at initial mannitol stress (18% to 29%) at 100-300 mM conc. as compared with cv. GK 909 where a significant decrease of 40.32-53.45% as observed, however, with the increase in conc. from 400-500 mM the decrease in the shoot length was marginal in both the cultivars. The decrease in the root length was however doesn't decreased significantly the both the cultivars. Relative water content decreased in both the cultivars during the drought stress period (Fig. 2). The RWC decreased from 76.78% and 73% in control to 62.45% and 52.55% at 500 mM conc. in GK 101 and GK 909 variety.

Results revealed that in cv. GK-101 with the increase in the stress condition from 100-500 mM, the MDA content initially increased in 100 mM conc. by 23.08 % followed by a decrease from 200-400 mM conc. by 7-17% before increasing to about 32.25% at 500 mM conc. with reference to control sample. Under similar conditions, the cv. GK-909 showed a slight decrease at 100 mM conc. before increasing at 200-500 mM conc. from 10-55% (Fig. 3).

As observed in Fig. 3, lipid peroxidation which was measured as MDA content, there was an increase at 100 and 500 mM conc whereas a decrease was observed at 200-400 mM stress condition in cv. GK 101. MDA content was 0.10 μgg^{-1} DM and 0.12 μgg^{-1} DM at 100 and 500 mM conc. and 0.09, 0.075 and.0796 μgg^{-1} DM at 200-400 mM mannitol conc. Similarly in cv. GK 909 increase in drought stress from 200-500 mM consistently increased the MDA conc except at 100 mM where a decrease was observed as compared to control.

There was an increase of 0.04 to 0.065 $\mu g g^{-1}$ DM of MDA content with increased severity of stress.

Effect of drought stress on antioxidant enzymes activity

Specific activity of peroxidase for G.K.101 showed a transient behavior (Fig. 4). The POD activities were increased by 17.10 %, 39.52% and 89.09 % due to 100 mM, 300 mM and 400 mM mannitol stress. The decrease was found for 500 mM mannitol stress. In G.K.909 revealed that the decrease in activity of 45.54 % and 3.71% induced as a result of 500 mM and 200 mM mannitol stress. The 400 mM sample showed 66.45% of highest activity than all the samples. The 100 mM mannitol induced 13.47% increase in activity but lesser than 300 mM sample. The lowest increase of 12.14% was observed for 400 mM sample.

The specific activity of catalase for G.K.101 revealed that CAT activity reduced by 40.42%, 41.76% and 76.15% at 200 mM, 300 mM and 500 mM under mannitol treatment (Fig. 5). The highest activity reduction was in 500 mM sample. The specific activity increased by 14.73% and 31.20 % at 100 mM and 400 mM than the control sample. There was a marked increase of 33.16% and 47.76% and 53.21% at 200-400 mM conc. in G.K.909 cultivar The 200 mM sample showed a constant decrease in CAT activity by 12.04%.

The 500 mM mannitol treatment in G.K.909 significantly increased the SOD activity and the highest 19.1% SOD activity was found in 500 mM sample than all other samples (Fig. 6). The 100 mM mannitol induced a 8.05% decrease of antioxidant activity. The activity value was increased by 2.77%, 11.38% and 7.38% in 200 mM, 300 mM sample and in 400 mM than the control sample. The specific activity of SOD was also assessed for G.K.101. The activity increased 2.68% under for 400 mM mannitol stress. The decrease of 14.15% was found in 100 mM sample. The activity was then increased upto 400 mM sample. The 500 mM mannitol reduced 2.68% of activity of enzyme.

Activity staining analysis of different antioxidative isozymes

The peroxidase bands were of high molecular weight protein which was present on the upper side (Fig 7). The high molecular weight proteins were of approximately same intensities so salinity stress did not affect the POD activity. The in gel assay of POD for G.K.101 were revealed nearly same results as that of G.K.909. But the bands of G.K.101 were of lower resolution as compared to G.K.909 (Fig. 7). The band 100 mM sample was more intense than control sample. The bands in 500 mM sample was a little lesser intense than other samples. But the difference of bands in the entire sample was very minute.

The cultivar G.K.909 showed sharp and high resolution bands were revealed for CAT. The intensity of bands was highest for 100 mM and 200 mM sample and lowest for 500 mM samples (Fig. 7). There was little difference in activity from control and 100 mM sample The activity for 200 mM sample was increased. Therefore the CAT activity was increased with increase in drought concentration. The in gel assay of CAT for G.K.101 revealed very intense and clear bands. The highest resolution bands were obtained for 400 mM sample (Fig. 7). The activity of CAT was then decreased for 500 mM sample as compare to 400 mM sample. The 100 mM samples showed the least intense bands and lowest activity of CAT.

The activity of superoxide dismutase showed a contrasting behavior in both the cultivars. It was

observed that there are intense bands at 100, 300 and 400 mM conc. and the banding pattern intensity was lower at 200 and 500 mM conc. in cv. GK-101 and almost a similar banding pattern was observed in case of cv. GK-909.

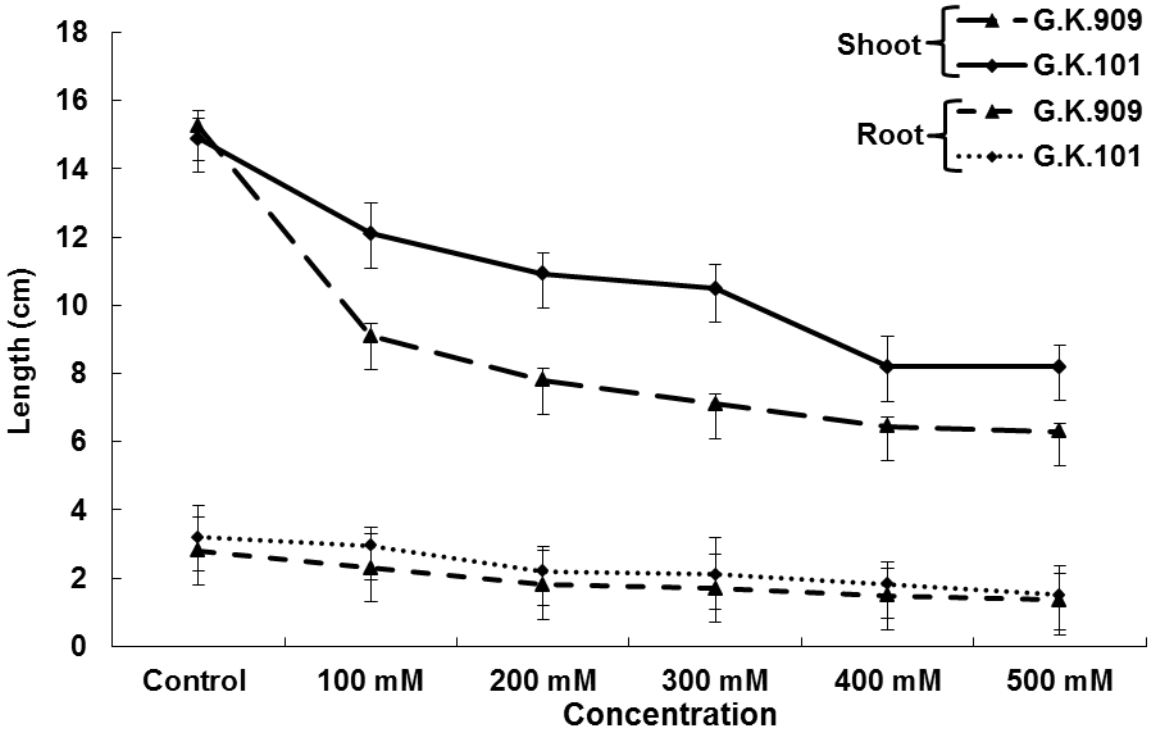

Figure 1. Effect of drought stress on root/shoot length in different sorghum cultivars.

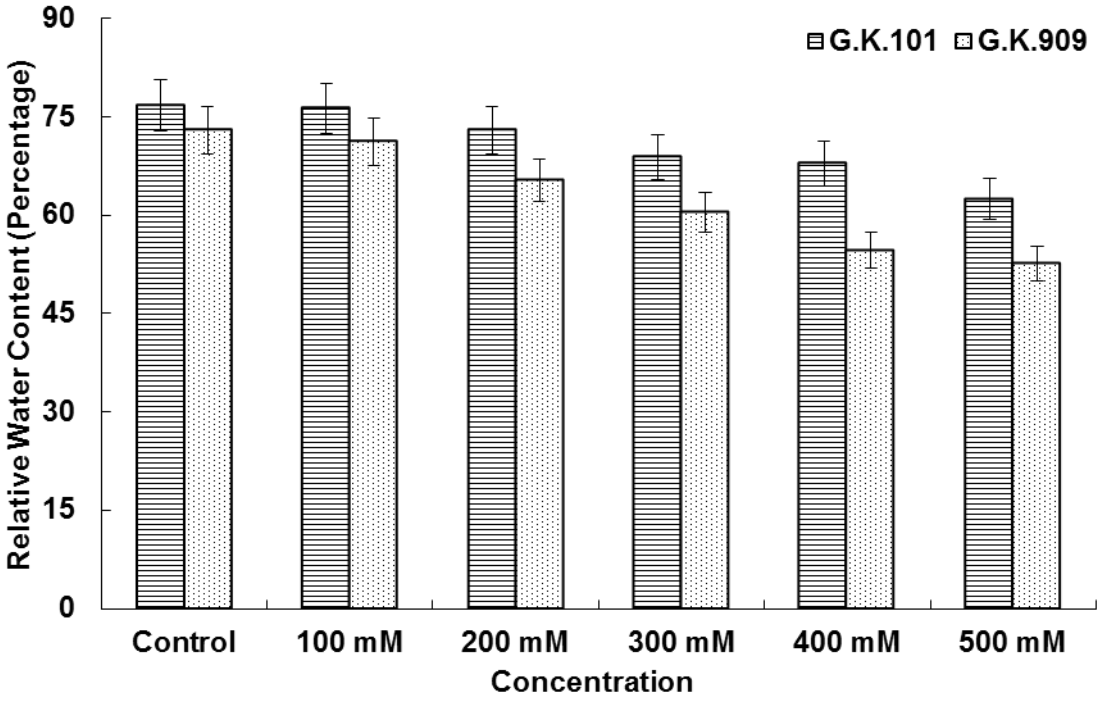

Figure 2. Comparative analysis of RWC in sorghum cultivars under water stress conditions. Error bars represent the standard deviation.

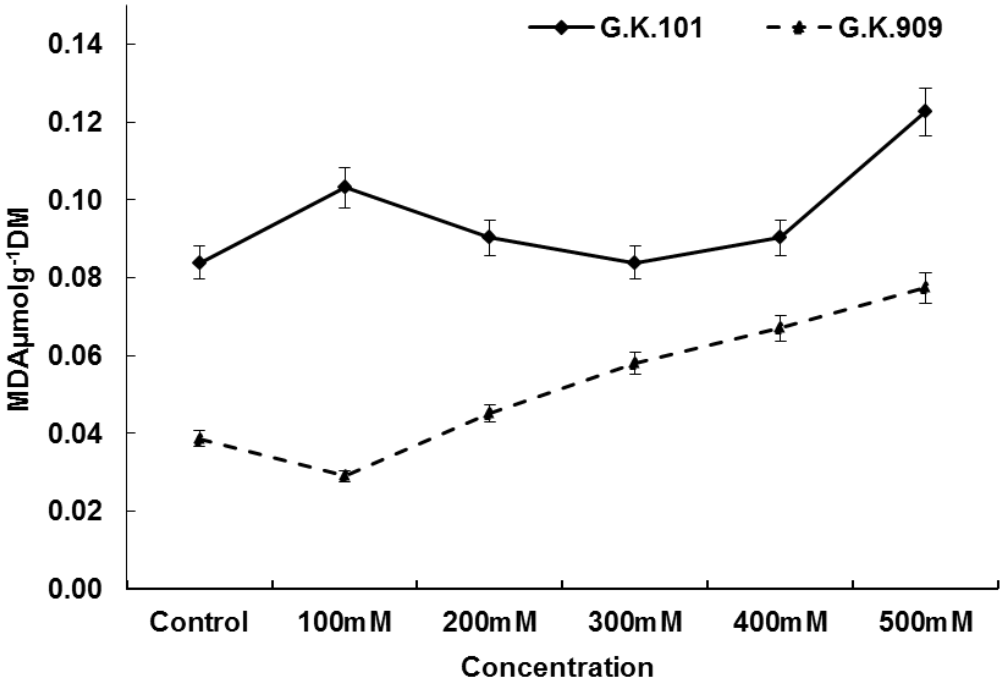

Figure 3. MDA analysis for determination of Lipid peroxidation in sorghum cultivars under water stress conditions.

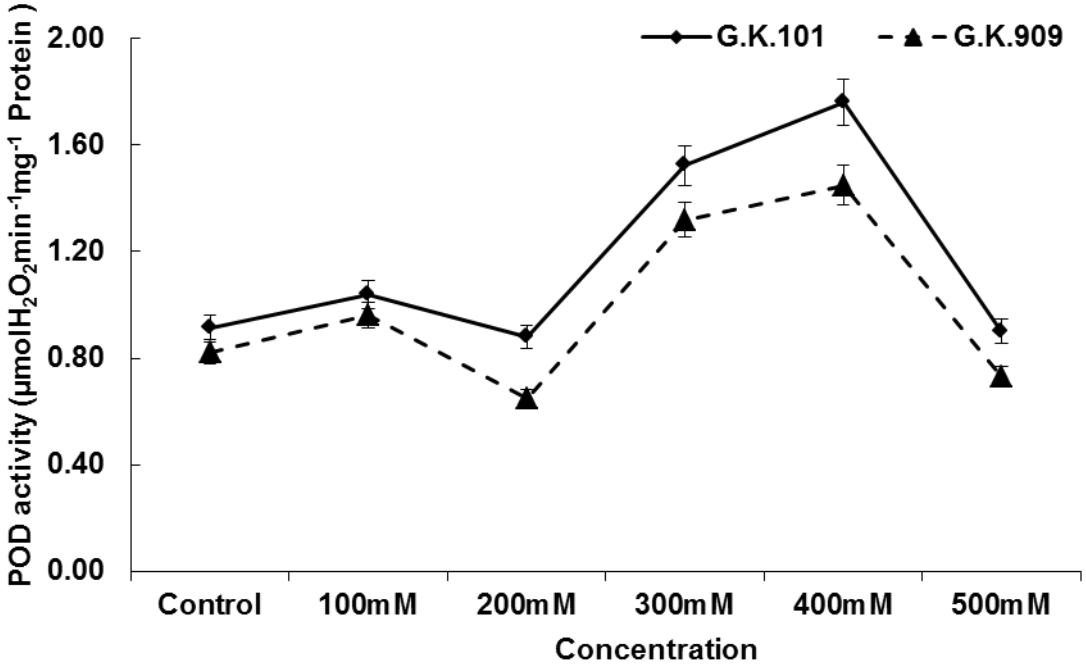

Figure 4. Catalase analysis in sorghum cultivars under water stress conditions.

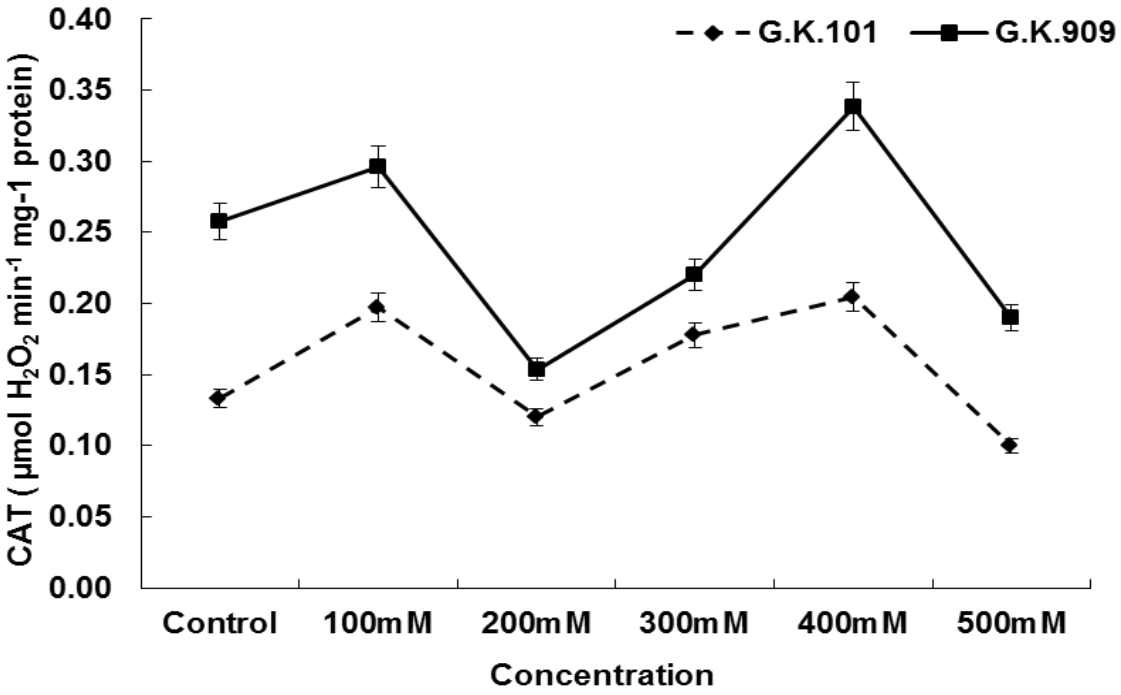

Figure 5. Peroxidase analysis in sorghum cultivars under water stress conditions.

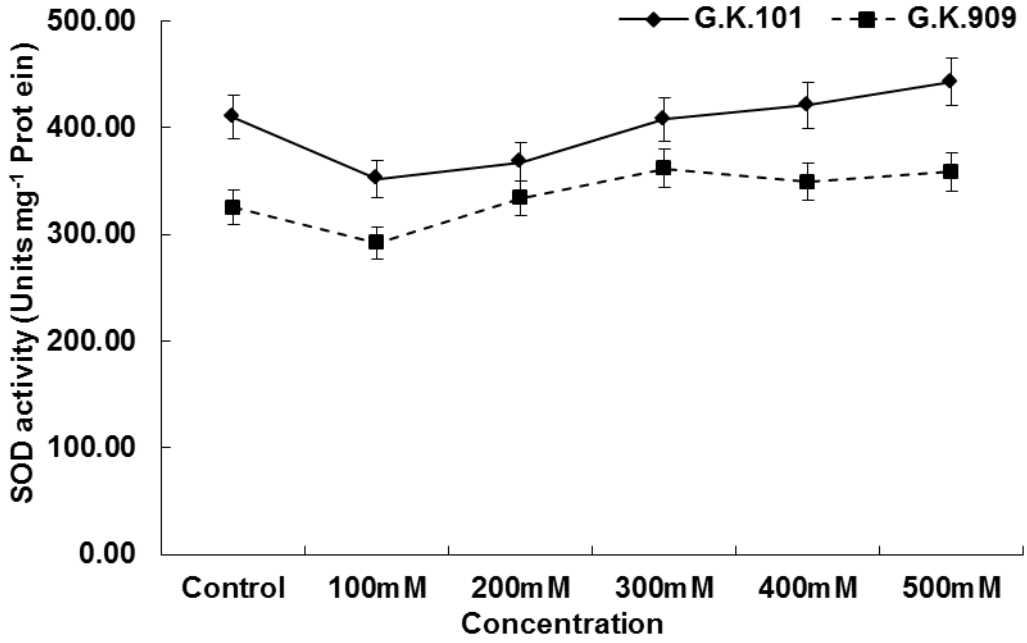

Figure 6. Superoxide dismutase analysis in sorghum cultivars under water stress conditions.

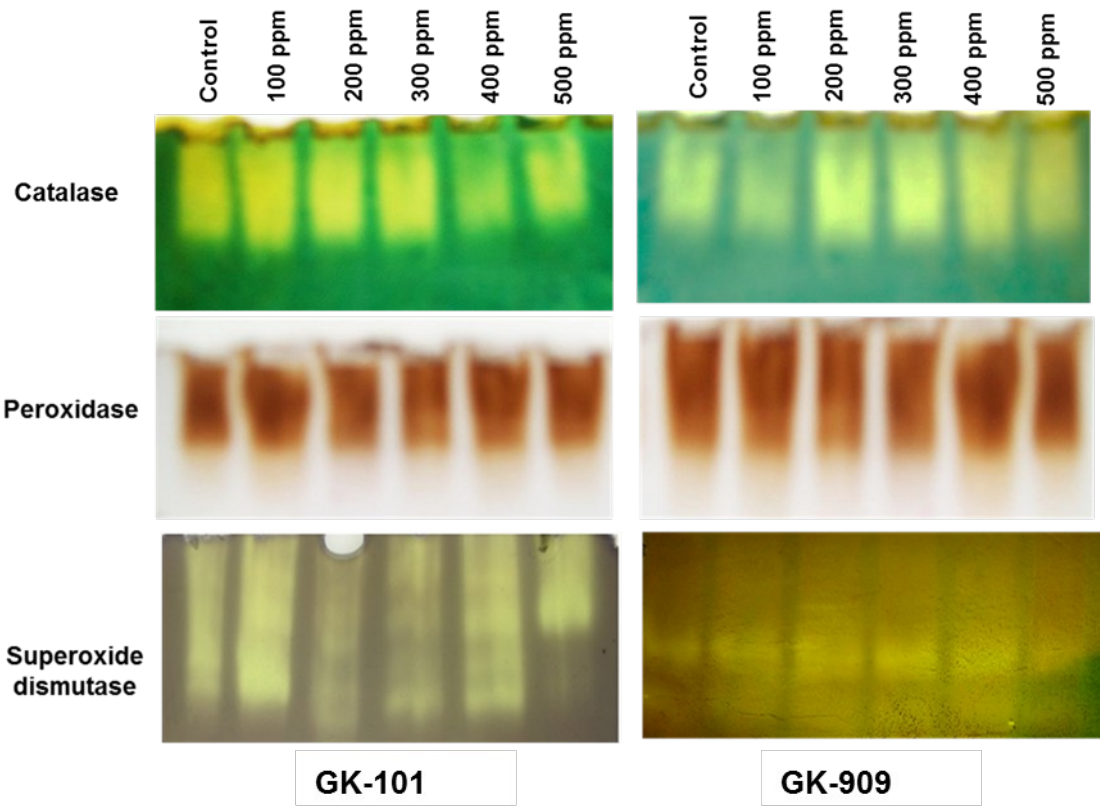

Figure 7. In-gel assay of catalase, superoxide dismutase and peroxidase isozymes in two cultivars of sorghum under drought stress.

DISCUSSION

We studied the role of mannitol stress on the physiological and antioxidative enzymes in sorghum cultivars. Under water stress the growth of shoot and root responded differently in the two cultivars. It was observed that a consistent decrease in the shoot tissue of the two cultivars was noted whereas a non-significant decrease was observed in the root tissues (Fig. 1). Growth arrest can be considered as a possibility to preserve carbohydrates for sustained metabolism, prolonged energy supply and for better recovery after stress relief. The inhibition of shoot growth during water deficit is thought to contribute to solute accumulation and thus eventually to osmotic adjustment (Osorio *et al*. 1998; Spollen *et al*. 2003). On the other hand, continuation of root growth under osmotic stress is an adaptive mechanism that facilitates water uptake from deeper soil layers (Munns, 2000).

The leaf relative water content directly reflects the water status of plants. It has been reported that the rate of RWC in plants with high resistance against drought is higher than others. Our results showed that the RWC decreased consistently in both the cultivars, though, the pattern differs. The decrease in RWC in plants may be due to the change in the cell membrane structure which is subjected to changes like increase in penetrability and decrease in sustainability (Blokhina *et al*., 2003). The leaf relative water content directly reflects the water status of plants as reported earlier (Marron *et al*., 2002; Liang *et al*., 2006).

Lipid peroxidation, which was known to be one of the important parameter to study the abiotic stress tolerance, was assessed by analyzing Malondialdehyde (MDA) content. This work revealed a transient behavior pattern of MDA in different cultivars of sorghum under stress. Since MDA is one of the end products of lipid peroxidation, the change in the MDA content reflects the degree of the peroxidation of membrane lipids (Taulavuori *et al*., 2001). The increase of MDA contents in response to drought stress in the sorghum species suggested drought stress caused oxidative damages in both two species, similarly as detected in olive trees (Sofo *et al*., 2004), sunflower (Bailly *et al*.,1996) and *Coffea arabica* (Queiroz *et al*., 1998).

Analysis of the antioxidant enzymes revealed that the both the cultivars have a constant behavior for different enzymes viz. POD, CAT and SOD. Plants have an internal protective enzyme-catalyzed clean up system, which is fine and elaborate enough to avoid injuries of active oxygen, thus guaranteeing normal cellular function (Horváth *et al*., 2007). The balance between ROS production and activities of antioxidative enzyme determines whether oxidative signaling and/or damage will occur (Moller *et al*., 2007). Maintaining a higher level of antioxidative enzyme activities may contribute to drought induction by increasing the capacity against oxidative damage (Sharma and Dubey, 2005). Our results indicated that the sorghum cultivars level of peroxidase, catalase and superoxide dismutase varied with the change in severity of stress in the different cultivars under study. It has been proved that efficient antioxidative characteristics can provide better protection against oxidative stress in leaves under drought stress (Reddy *et al*., 2004).

In response to unfavourable conditions, most plants will activate their stress coping mechanisms such as acclimation of metabolic fluxes, activation of repair processes and long-term metabolic and morphological adaptations (Lichtenthaler, 1996). Such mechanisms include *de novo* synthesis of proteins with specific adaptive functions, osmotic adjustment, antioxidative defense, among others. Electrophoretic analysis was carried out to study the behavior of different antioxidative enzymes under the influence of mannitol stress. Our results revealed a similar behavioral pattern of POD and CAT activity in response to drought stress various plants as reported with other plant species like sunflower (Gunes *et al*., 2008), poplar (Xiao *et al*., 2008), brassica species (Das and Uprety, 2006) and wheat (Csiszar *et al*., 2005).

The activity of superoxide dismutase showed a contrasting behavior in both the cultivars. It was observed that there are increase in the activity of SOD with the increase in the stress. An increase in the SOD activity may be attributed to the increased production of active oxygen species as substrate that lead to increased expression of genes encoding SOD. Our results are consistent with other studies reporting the increased SOD activity in response to drought stress in sunflower (Gunes *et al*., 2008), cowpea (Manivannan *et al*., 2007) etc. These results thus manifest that acclimation to drought stress has been associated with the rapidity, severity, duration of the drought event and their interaction and can be used as biomarker for stress studies.

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