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EFFECT OF DROUGHT STRESS ON LIPID PEROXIDATION AND PROLINE CONTENT IN COTTON ROOTS

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ABSTRACT

Oxidative damage and antioxidant responses were compared in two cotton cultivars: drought-resistant (CCRI-60) and drought-sensitive (CCRI-27). Cotton seedlings were subjected to PEG-6000 solutions with two different osmotic potentials, and changes in the antioxidant enzyme activities were investigated. Increasing PEG concentration reduced root length and vigor, increased H_2O_2, malondialdehyde (MDA), and proline contents, decreased or showed no change in antioxidant enzyme levels and increased glutathione reductase activity in CCRI-27. On the other hand, CCRI-60 showed increased root length and vigor, no substantial variation in MDA content, increased antioxidant enzyme activities, and significantly increased glutathione reductase (GR) activity and proline content. Differences in the activation of antioxidant defense systems suggested a role for activated oxygen species in the cellular toxicity of drought stress. Overall, the cultivar CCRI-60 was better able to resist drought as indicated by better growth due to the higher free radical scavenging capacity, and better protection mechanism. Therefore, this results could be used to select or create new varieties of cotton plants to obtain a better productivity under water deficit conditions.

Keywords: Antioxidant enzymes; malondialdehyde; PEG concentration; reactive oxygen species; osmotic potential

INTRODUCTION

Cotton, the leading global natural fiber source, is grown in both arid and semi-arid regions worldwide (Iqbal *et al*., 2012). Drought is one of the major yield-limiting factors for cotton crops (Anjum *et al.*, 2012). Soil water deficiency is known to significantly affect plant growth (Li *et al.*, 2001; Liu *et al.*, 2004). Roots function as sensors and detect soil dehydration, possibly by alteration of their water status; thus, they play a role in plant resistance to water shortage (Gao *et al.*, 2014). Therefore, improving our understanding of the interactions between root function and drought could have a significant impact on global cotton production.

Alterations in antioxidant enzyme metabolism could influence drought tolerance of cotton (Deeba *et al.*, 2012). Drought stress promotes the production of reactive oxygen species (ROS), which can be detrimental to proteins, lipids, carbohydrates, and nucleic acids (Dietz and Pfannschmidt, 2011). Plants have both enzymatic and nonenzymatic defense systems for scavenging and detoxifying ROS. Superoxide dismutase (SOD) scavenges O_2^- to H_2O_2 (Ghobadi*et al.*, 2013). Peroxidase (POD), catalase (CAT), ascorbate peroxidase (APOX), and glutathione reductase (GR) decompose H_2O_2 into H_2O at different cellular locations (Mittler, 2002). In the absence of natural scavengers such as CAT and POD, high levels of H_2O_2 accumulate in tissues. APOX, along with monodehydroascorbatereductase (MDHAR), dehydroascrobatereductase (NADH), and GR, removes H_2O_2 via the Halliwell–Asada pathway (Uzilday *et al.*, 2012). APOX reduces H_2O_2 to water by oxidizing ascorbate to MDHA (André *et al.*, 2006) and thus plays a key role in the ascorbate-glutathione cycle. MDHA is then reduced to ascorbate by MDHAR. Nonetheless, 2 molecules of MDHA can be nonenzymatically converted to MDHA and dehydroascorbate, which is then reduced to ascorbate via the dehydroascorbatereductase and GR cycle (Szalai *et al.*, 2009). In this cycle, GR oxidizes reduced glutathione (GSH) to oxidized glutathione (GSSG). The balance between ROS production and antioxidative enzyme activities determines whether oxidative signaling and/or damage will occur.

The responses of CAT, APOX, and GR activities to osmotic stress remain controversial. The effects of water stress on enzyme activities are disputable and depend on both the degree of tolerance of plants and the specific mechanism underlying water stress (Kaur*et al.*, 2013). Maintaining high levels of antioxidative enzyme activities may contribute to drought tolerance by affording better protection mechanisms against oxidative damage (Devi *et al.*, 2012). Therefore, comparing antioxidant defense systems, lipid peroxidation levels, and proline contents in roots of cotton cultivars differing in drought tolerance might allow a better understanding of tolerance mechanisms to drought stress. We hypothesized that increased constitutive or induced antioxidant enzyme activities in the roots of cotton plants might afford tolerance to short-term drought stress. This

study aimed to evaluate the mechanism underlying adaptation to drought stress in the roots of two cotton cultivars differing in drought resistance.

MATERIALS AND METHODS

Experimental design: The seeds of twocotton cultivars-CCRI-60 (drought-resistant) and CCRI-27 (drought-sensitive) were obtained from the National Medium-Term Gene Bank of Cotton in China and soaked in sterile deionized water at 28°C for 6 h. Next, they were transferred to two sheets of sterile filter paper moistened with deionized water and placed in plastic trays for germination at 28°C for 72 h in the dark. Germinated seeds were sown into holes in Styrofoam boards placed in deionized water and grown hydroponically in a growth room for three weeks under fluorescent and incandescent lights. The growth room was maintained at 27 ± 2°C, with photosynthetically active radiation level of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and daytime humidity of 60–70%.

After three weeks, healthy and uniform seedlings were selected and inserted in holes in circular polystyrene lids, which were fixed on 24 pots containing aerated Hoagland nutrition solution (pH 5.2). The nutrition solution was replaced twice a week. The seedlings were acclimated to the hydroponic culture conditions for one week and submitted to different drought stress treatments produced by adding, in a single step, 0% PEG 6000, 10% PEG 6000, and 20% PEG 6000 to the nutrient solution to obtain osmotic potentials of approximately 0, -0.52 MPa, and -0.77 MPa, respectively. Controls were grown in untreated nutrient solution. After 7 days of water stress, the roots were immediately washed with distilled water, blotted dry on a filter paper, and stored at -20°C until enzyme analyses.

From each group, 20 plants randomly divided into separate leaf and root fractions. Root fresh weights (FWs) were then recorded, and root lengths were measured. The samples were then dried in an oven at 80°C for 72 h, and dry weights were determined.

Measurements and methods: Frozen leaf segments (0.5 g) were crushed into a fine powder using a mortar in an ice bath. Approximately 5.0 mL of 0.05 $mol \cdot L^{-1}$ phosphate buffer (pH 7.8) with 1% polyvinylpyrrolidone (PVP) was used as the extraction buffer. The homogenate was centrifuged at 15,000 ×*g* for 15 min at 4°C, and the supernatant was used to measure malondialdehyde (MDA) content according to the method of Zhang (1992).

H_2O_2 content was estimated according to methods of Bernt and Bergmeyer (1974). Approximately 0.5 g of roots from control and treatment groups were homogenized with lipid nitrogen, and the powders were suspended in 1.5 mL of 100 mM potassium phosphate buffer (pH 6.8). The suspensions were then centrifuged at 18,000 ×*g* for 20 min at 4°C. The enzymatic reaction was initiated with 0.25 mL supernatant and 1.25 mL peroxidase reagent, consisting of 83 mM potassium phosphate buffer (pH 7.0), 0.005% (w/v) *O*-dianizidine, and 40 µg peroxidase/mL at 30°C. The reaction was stopped after 10 min by adding 0.25 mL of 1 N perchloric acid, and the reaction mixture was centrifuged at 5000 ×*g* for 5 min. The absorbance of the supernatant was read at 436 nm, and the amount of H_2O_2 was determined using an extinction coefficient of 39.4 $mM^{-1} \cdot cm^{-1}$.

Frozen root samples (0.3 g) were crushed into a fine powder using a mortar in an ice bath. SOD activity was determined according to methods of Foster and Hess (1980). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of nitrobluetetrazolium (NBT) reduction, which was measured spectrophotometrically (UV-2401; Shimadzu Corporation, Japan) at 560 nm. Total CAT activity was measured according to the method reported by Jr and Sizer (1952), with minor modifications. The reaction mixture (1.5 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20mM H_2O_2, and 50 µL enzyme extract. The reaction was initiated by the addition of the enzyme extract. The decrease in H_2O_2 was monitored at 240 nm and was quantified by its molar extinction coefficient (36 $M^{-1} \cdot cm^{-1}$). POD activity was analyzed in 2.9 mL of 0.05 $mol \cdot L^{-1}$ phosphate buffer, containing 1.0 mL of 0.05 $mol \cdot L^{-1}$ guaiacol and 1.0 mL of 2% H_2O_2 (Tan *et al.*, 2008). Increases in absorbance at 470 nm were recorded after adding 2.0 mL of 20% chloroacetic acid.

APOX (EC 1.11.1.11) activity was determined according to the method reported by Nakano and Asada (1981). The reaction mixture contained 0.05 M Na-phosphate buffer (pH 7.0), 0.5 mMascorbate, 0.1 mM EDTA Na_2, 1.2 mM H_2O_2, and 0.1 mL enzyme extract in a final assay volume of 1 mL.Ascorbate oxidation was analyzed at 290 nm.

GR (EC1.6.4.2) activity was measured as described by Foyer and Halliwell (1976). The assay medium contained 0.025 mM Na-phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 Mm NADPH Na_4, and 0.1 mL extract enzyme in a final assay volume of 1 mL. NADPH oxidation was analyzed at 340 nm. The specific enzyme activity for all enzymes was expressed in terms of units$\cdot mg^{-1}$ protein$\cdot g^{-1}$ FW.

Free proline content was analyzed using previously published methods (Bates *et al.*, 1973). Root samples (0.5 g) from each group were homogenized in 3% (w/v) sulfosalicylic acid, and the homogenates were filtered through a filter paper. Acid ninhydrin and glacial acetic acid were added, and the resulting mixture was heated to 100°C for 1 h in a water bath. The mixture was extracted with toluene, and the absorbance of the fraction with toluene that was aspired from the liquid phase was read at 520 nm.

Data analysis: All experiments were repeated twice independently, and each data point represents the mean of 3 replicates (n = 6), except for root length data of the cotton seedlings (n = 20). All data obtained were subjected to ANOVA, and the mean difference was compared by the LSD test at 95% or 99% levels of probability. In all the figures, the spread of values is shown as error bars representing standard errors of the means.

RESULTS AND DISCUSSION

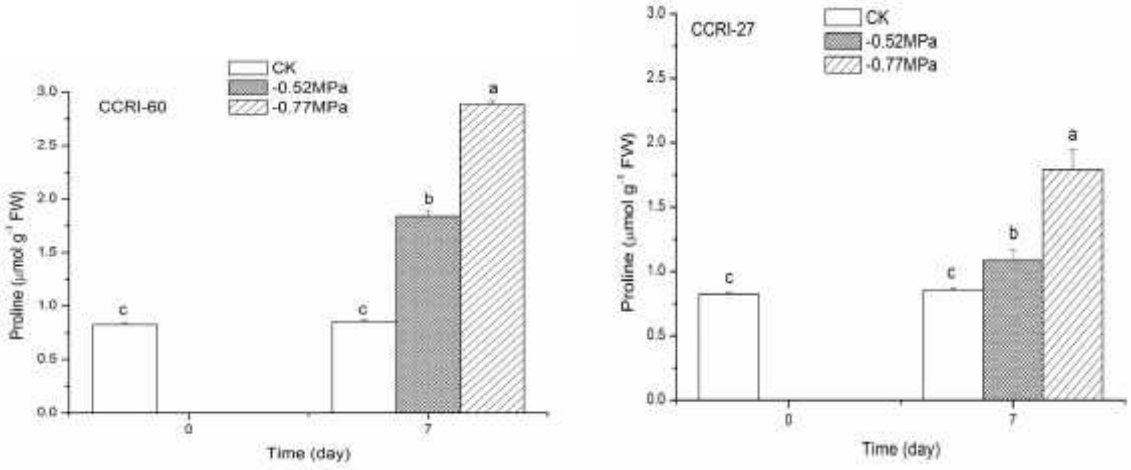
Breeding studies advocate the selection for a deep and extensive root system to increase the productivity of food legumes under moisture-deficient conditions for optimizing the plants' capacity to acquire water (Subbarao*et al.* 1995). In the present study, although root FW decreased with increasing PEG concentration, drought stress had no significant effect on FW in either cultivar (Table 1). Root length of both cultivars from treated plants was lesser as compared to that of the control. However, this reduction was only significant in CCRI-27 ($p \leq 0.05$) grown in PEG solutions with osmotic potentials of -0.52 MPa and -0.77 MPa. Root vigor increased concomitantly with increasing concentrations of PEG for CCRI-60, while CCRI-27 showed the opposite trend. The decrease in root vigor in CCRI-27 was significant ($p< 0.05$) at an osmotic potential of -0.77 MPa, but not at -0.52 MPa. However, Lei *et al.* (2006)proposed that plants can utilize a limited water resource more efficiently by increasing the water-absorbing root biomass, which was noted in CCRI-60 that retained higher root lengths and root vigor under stress conditions. The CCRI-27 cultivar might have reduced biomass allocation to roots under drought conditions.

Table 1. Effect of PEG treatment on the fresh weight (FW), root length and root vigor of two cotton cultivars

Cultivars	Parameter	Day 0	Day 7 :Osmotic potential of PEG solution(MPa)		
			0	-0.52	-0.77
CCRI-66	FW(g)	0.176±0.005b	0.212±0.006a	0.204±0.006a	0.199±0.011a
	Root length(cm)	19.69±0.74081b	31.74±0.54a	31.33±0.20a	31.20±0.13a
	Root vigor	320.97±3.06b	311.74±0.54c	322.46±2.42b	326.87±1.52a
CCRI-27	FW(g)	0.132±0.006b	0.150±0.007a	0.149±0.003a	0.143±0.004a
	Root length(cm)	19.26±1.71d	29.68±0.39a	28.58±0.48b	27.27±0.66c
	Root vigor	320.74±4.28a	309.58±9.81ab	299.92±7.39b	279.60±6.27c

Each value indicated is the mean of six replicated recorded ± S.E. Means followed by the same letters are not significantly different at P< 0.05 within the same cultivar.

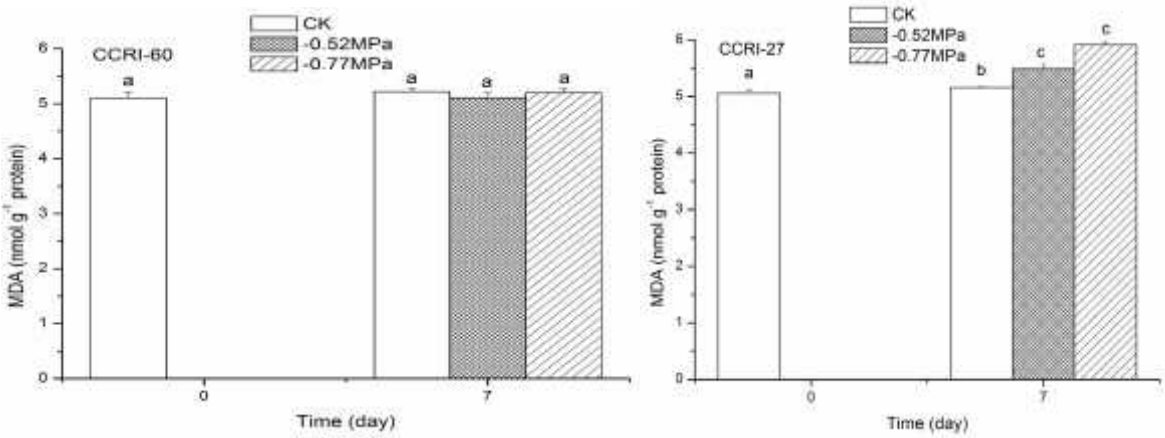
The free proline content increased significantly ($p< 0.05$) in the roots of both cultivars with increasing PEG concentration (Fig. 1). Furthermore, the proline content was higher in CCRI-60 than in CCRI-27, particularly at -0.77 MPa osmotic potential. Proline accumulation plays adaptive roles in plant stress tolerance and is known to be the first response of plants exposed to water-deficit stress (Anjum *et al.*, 2011).



Values are the means of six replicated ±S.E. Bars with different letters are significantly different at P<0.05

Fig. 1. Effect of PEG treatments on proline content in roots of CCRI-60 and CCRI-27

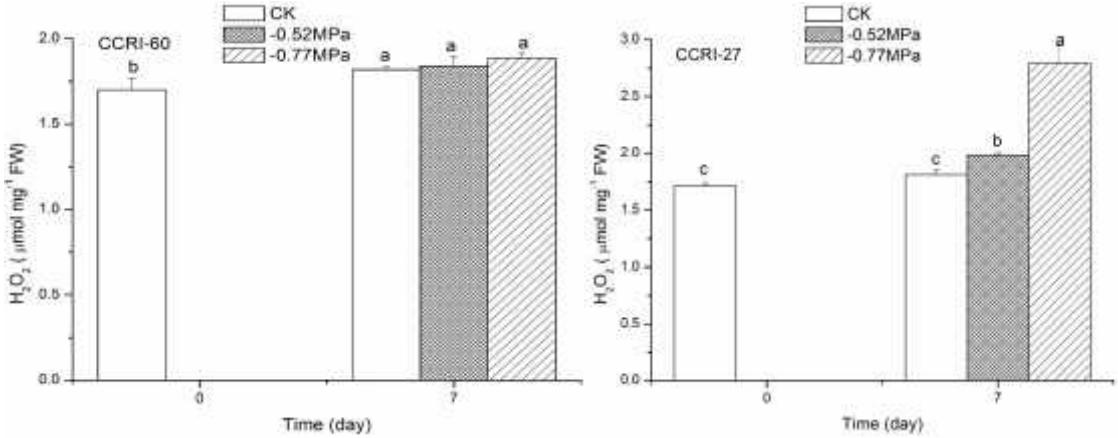
The free radical-induced peroxidation of lipid membranes is known to reflect stress-induced damage at the cellular level (Shukla *et al.,* 2012). A small "age-dependent" increase in lipid peroxidation levels was evident after 7 days in the roots of both cultivars growing under normal growth conditions, which was statistically insignificant for CCRI-60; however, there was a gradual increase in the lipid peroxidation level of CCRI-27 at osmotic potentials of -0.52 MPa and -0.77 MPa (Fig. 2). Unlike the results for CCRI-27, the roots of CCRI-60 showed no significant change in lipid peroxidation with increasing PEG concentration. This finding suggested that CCRI-60 may have better protection against oxidative damage under drought stress. The level of MDA, which produced during peroxidation of membrane lipids, was used as an indicator of oxidative damage.



Values are the means of six replicated ±S.E. Bars with different letters are significantly different at P<0.05

Fig. 2. Effect of PEG treatment on malondialdehyde (MDA) content in roots of CCRI-60 and CCRI-27

H_2O_2 content was higher in CCRI-27 than in CCRI-60, irrespective of experimental conditions (Fig. 3). H_2O_2 content did not change significantly in CCRI-60 roots with increasing PEG concentration. This could be attributed to the efficient removal of H_2O_2 by the increased activities of CAT, POD, and SOD. On the other hand, H_2O_2 content increased significantly in CCRI-27 roots, particularly at -0.77 MPa osmotic potential. This could be due to the decreased CAT activities, which could result in a Haber–Weiss reaction to form hydroxyl radicals as described by Bowler *et al*. (1992).

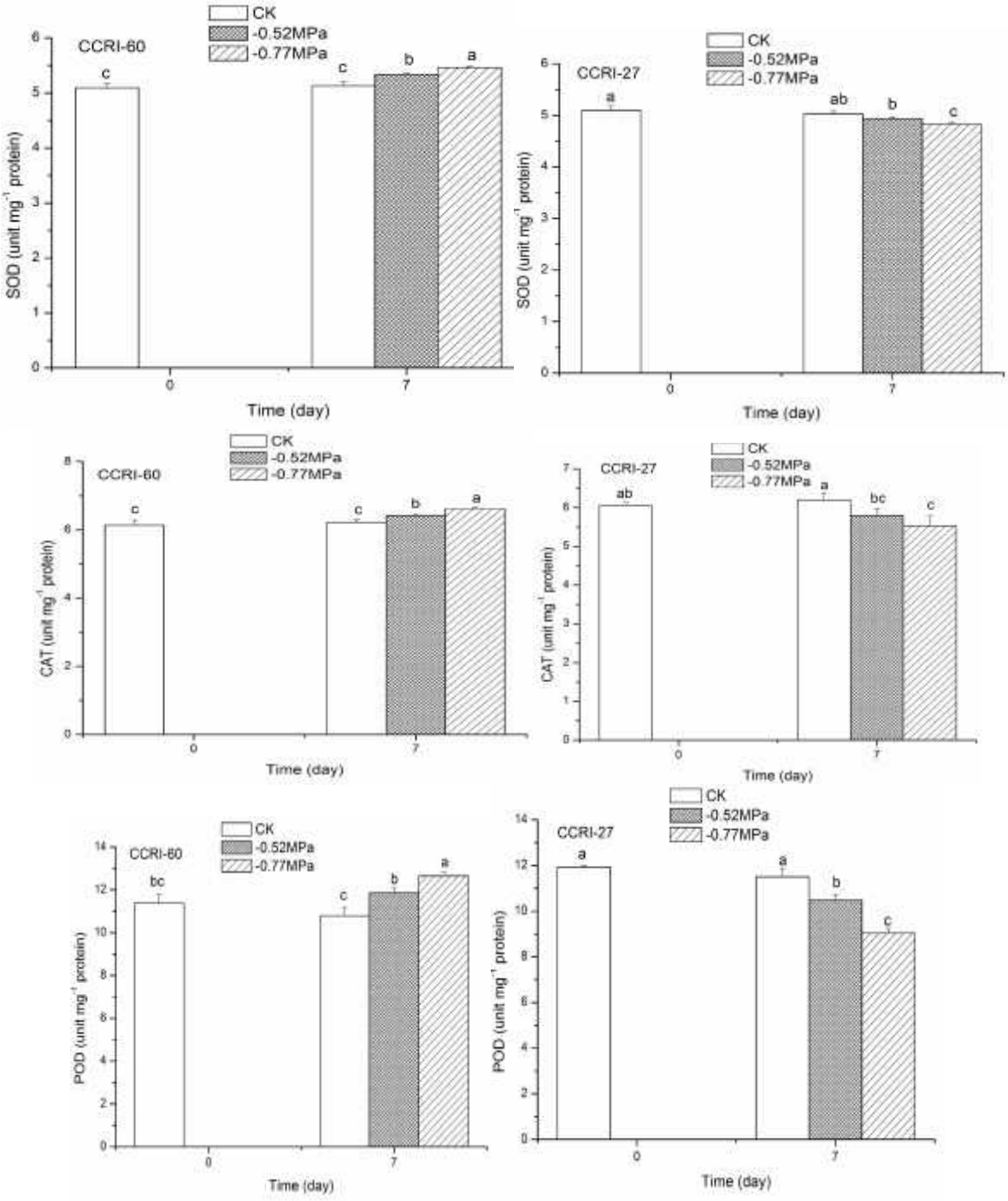


Values are the means of six replicated ±S.E. Bars with different letters are significantly different at P<0.05

Fig. 3. Effect of PEG treatments on H_2O_2 content in roots of CCRI-60 and CCRI-27

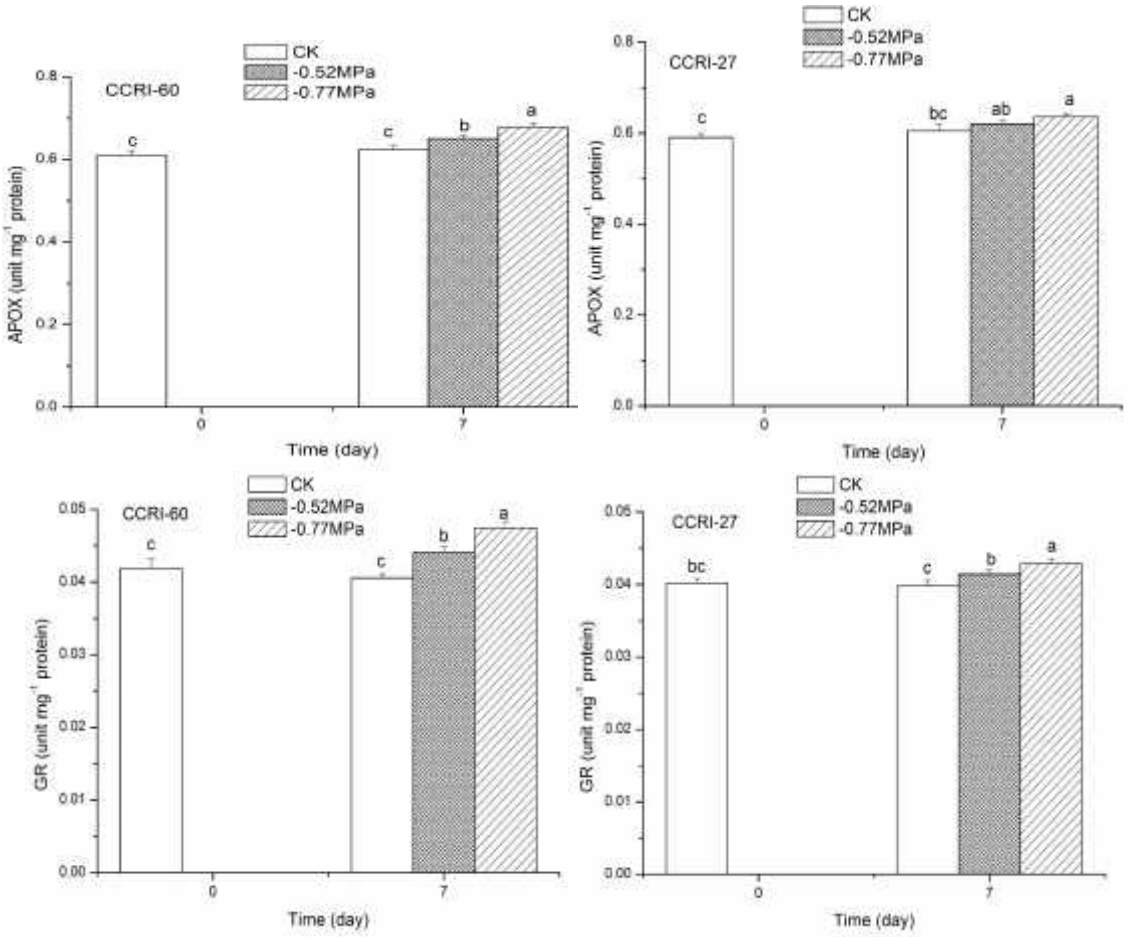
Plants efficiently decompose ROS by using antioxidative enzymes (Luis *et al.*,2014). There were striking differences in the antioxidant enzyme activities between both the cultivars with increasing PEG concentrations. Although the roots of both cultivars showed remarkable changes in SOD, POD, and CAT activities with increased drought stress, these activities increased in CCRI-60 roots but decreased in CCRI-27 (Fig. 4). APOX activity

increased slightly in CCRI-60 roots but did not change significantly in CCRI-27 roots after 7 days of exposure to increasing PEG concentrations (Fig. 5).



Values are the means of six replicated ±S.E. Bars with different letters are significantly different at P<0.05

Fig. 4 Effect of PEG treatments on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) activity in roots of CCRI-60 and CCRI-27



Values are the means of six replicated ±S.E. Bars with different letters are significantly different at P<0.05

Fig.5. Effect of PEG treatment on ascorbate peroxidase (APOX), and glutathione reductase (GR) activity in roots of CCRI-60 and CCRI-27

Our findings suggested that drought stress induces the expression of antioxidant enzymes in the drought-resistant cultivar CCRI-60. Hence, CCRI-60 could cope well with a lower magnitude of drought stress by increasing the activities of mainly SOD, POD, CAT, and, to some extent, APOX. Since CAT and APOX are dominant in the roots of CCRI-60, coordinated activities of these enzymes might act as effective antioxidative defenses against the effect of H_2O_2 under drought conditions.

Similar to the findings of the study by Acar *et al.* (2001), the drought-sensitive cultivar CCRI-27 was affected more by drought and exhibited lower POD activity in present study. Low POD activity under water deficit could be regarded as a precondition for drought sensitivity. GR activity significantly ($p< 0.05$) increased in the roots of both cultivars with increasing PEG concentration. The CCRI-60 showed a greater increase in GR activity than CCRI-27 at both osmotic potentials (Fig. 5). Consistent with our results, Luis *et al.*(2014)noted increased GR activity in the leaves of both drought-resistant and drought-sensitive sugarcanegenotypes under drought stress. Besides removing H_2O_2, GR activity resulted in the availability of NADP, which can accept electrons from ferrodoxin, thereby minimizing the chances of superoxide formation (Arora *et al.*, 2002).

Conclusions: The SOD-POD-CAT system was found to protect cotton plants from oxidative damage during drought stress, individual activities of these enzymes differed substantially in two cotton cultivars. The higher free radical scavenging capacity and better protection mechanism of CCRI-60 against drought stress were due to the lower level of lipid peroxidation. These results confirmed that significant differences between the cotton cultivars in response to drought stress are closely related to differences in antioxidant enzyme activities.

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REFERENCES

Acar, O.,I. Tiirkan and F. Ozdemir (2001). Superoxide dismutase and peroxidase activities in drought sensitive and resistant barely (*Hordeum vulgate* L.) varieties.Acta Physiol. Plant.23: 351-356.

André, D.A.N., T.P. José, E.F. Joaquim, E.B. de A. Carlos and G.F. Enéas (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation on leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56: 87-94.

Anjum, S. A., M. F. Saleem, M. A. Cheema, M. F. Bilal and T. Khaliq (2012). An assessment to vulnerability, extent, characteristics and severity of drought hazard in Pakistan. Pakistan J. Sci. 64(2): 138-145.

Anjum, S.A., L.C. Wang, M. Farooq,M. Hussain,L.L. Xue andC.M. Zou (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J. Agron. Crop Sci. 197: 177-185.

Arora, A., R.K. SairamandG.C. Srivastava (2002). Oxidative stress and antioxidative system in plants. Curr. Sci. 82: 1227-1238.

Bates, L.S.,R.P. WaldrenandI.D. Teare (1973). Rapid determination of free proline for water-stress studies. PlantSoil39:205-207.

Bernt, E. and H.U. Bergmeyer (1974). Inorganic peroxidases. In: Bergmeyer HU (ed) methods of enzymatic analysis, 4. Academic press, New York, 2246-2248.

Bowler, C., M.V. Montagu and D. Inze (1992). Superoxide dismutase and salt stress tolerance. Ann. Rev. Plant Physiol. 43:83-116.

Deeba, F., A.K. Pandey, S. Ranjan, A. Mishra, R. Singh, Y.K. Sharma, P.A. Shirke and V. Pandey (2012). Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress.Plant Physiol. Bioch. 53: 6-18.

Devi, R., N. Kaur and A.K. Gupta (2012). Potential of antioxidant enzymes in depicting drought tolerance of wheat (*Triticum aestivum* L.). India J. Bioch. and Biophys. 49: 257-265.

Dietz, K.J. and T. Pfannschmidt (2011). Novel regulators in photosynthetic redox control of plant metablism and gene expression. Plant Phyiol. 155: 1477-1485.

Foster, J.G. and J.L. Hess (1980). Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. Plant Physiol.66: 482-487.

Foyer, C.H. andB. Halliwell (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metablism. Planta133: 21-25.

Gao, Y.H., Y.P. Xie, H.Y. Jiang, B. Wu and J.Y. Niu (2014). Soil water status and root distribution across the rooting zone in maize with plastic film mulching. Field Crop Res. 156: 40-47.

Ghobadi, M., S. Taherabadi, M.E. Ghobadi, G.R. Mohammadi and J.H. Saeid (2013). Antioxidant capacity, phtotsynthetic charateristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. Ind. Crop. Prod. 50: 29-38.

Iqbal, J., S. A. Wajid, A. Ahamd and M. Arshad (2012). Comparative studies on seed cotton yield in relation to nitrogen rate and sowing dates under diverse agro-environment of Punjab. Pakistan J. Sci. 64(1): 59-63.

Jr, R.F.B. andI.W. Sizer (1952). A spectrophotometric method of measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem.195：133-140.

Kaur, K., N. Kaur, A.K. Gupta and Singh (2013). Exploration of the antioxidative defense system to characterize chickpea genotypes showing differential response towards water deficit conditions. Plant Growth Regul. 70: 49-60.

Lei, Y.B.,C.Y. Yin and C.Y. Li (2006). Differences in some morphological, physiological and biochemical responses to drought stress in two contrasting populations of *Populus Przewalskii.* Plant Physiol. 127: 182-191.

Li, F.M., Q.H. Song,H.S. Liu,F.R. Li and X.L. Liu (2001). Effects of pre-sowing irrigation and phosphorus application on water use and yield of spring wheat under semi-arid conditions. Agr. Water Manage. 49:173-183.

Liu, H.S., F.M. Li and H. Xu (2004). Deficiency of water can enhance root respiration rate of drought-sensitive but not drought-tolerant spring wheat. Agr. Water Manage.64: 41-48.

Luis, F.B., C. Giselle, B. Lucélia, C. Silvana, G.A.L. Marcos, M. Paulo and A.A. Ricardo (2014). Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. Plant Physiol. Bioch. 74: 165-175.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. TrendsPlant Sci. 7: 405-410.

Nakano, Y. and K. Asada (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol.22:867-880.

Shukla, N., R.P. Awasthi, L. Rawat and J. Kumar (2012). Biochemical and physiological responses of rice (O*ryza sativa* L.) as influencced by *Trichoderma harzinaum* under drought stress. Plant Physiol. Bioch. 54: 78-88.

Subbarao, G.V., C. Johansen, A.E. Slinkard, R.C.N. Rao, N.P. Saxenaand Y.S. Chauhan (1995). Strategies and scope for improving drought resistance in grain legumes. Crit. Rev. Plant Sci.14: 469-523.

Szalai, G., T. Kellos, G. Galiba and G. Kocsy (2009). Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. J.Plant Growth and Regul.28: 66-80.

Tan, W., J. Liu,T. Dai, Q. Jing ,W. Cao andD. Jiang (2008). Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis waterlogging. Photosynthetica46: 21-27.

Uzilday, B., I. Turkan, A.H. Sekmen, R. Ozgur and H.C. Karakaya (2012). Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C_4) and *Cleome spinosa* (C_3) under drought stress. Plant Sci. 182: 59-70.

Yang, J.C., H. Zhang and J. H. Zhang (2012). Root morphology and physiology in relation to the yield formation of rice. J. Integ. Agr. 11(6): 920-926.

Zhang, X.Z. (1992). Crop physiological research methods. China Agricultural Press, Beijing. 131-207p (in Chinese).