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Research article

RESPONSE OF ANTIOXIDATIVE ENZYMES AND LIPOXYGENASE TO DROUGHT STRESS IN FINGER MILLET LEAVES *(ELEUSINE CORACANA (L.) GAERTN)*

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ABSTRACT: Millets are minor cereals of the grass family, Poaceae. Ragi or finger millet (Eleusine coracana L) is one of the common millets in several regions of India. The productivity of crop is greatly affected by various environmental stress. The current study presents the impact of drought stress on various antioxidant enzymes and on Lipoxygenase activity during finger millet plant growth (drought-tolerant, Sri Chaitanya VR-847). The possible involvement of activated oxygen species in drought stress was studied in finger millet leaves. The changes in H_2O_2, free proline content, and the rate of lipid peroxidation level in terms of malondialdehyde (MDA) in millet leaves was investigated in response to drought tolerance. Increased levels of MDA and higher amount of proline levels were observed under drought stress in the leaves. Antioxidant enzymes such as SOD, CAT, APX and GR showed elevated activities with drought stress in millet leaves. Lipoxygenases involved in many physiological and pathological activities and produces the reactive oxygen species by oxidizing unsaturated fatty acids which may be lethal to plant if present in excess. A significant increase in Lipoxygenase enzyme activity and its gene expression observed with water deficit condition in millets. The results indicate that improved tolerance to drought stress in millet crop may accomplished by increased capacity of lipoxygenase and antioxidative system for better drought tolerance in this cultivar.

Key words: Finger millet, Drought stress, Lipoxygenase, Antioxidative enzymes, Lipid peroxidation.

Abbreviations used:
LOX: Lipoxygenase; PUFA: Poly Unsaturated Fatty Acid; TCA: Trichloro acetic acid; MDA: Melanaldehyde; PMSF: Phenylmethylsulfonyl fluoride; TBA: Thiobarbituric acid; RT-PCR: Real time polymerase chain reaction; NBT: Nitro-blue tetrazolium; APX: Ascorbate peroxidase; SOD: Superoxide dismutase; CAT: Catalase; GR: Glutathione reductase; H_2O_2: Hydrogen peroxide; $O_2.-$: Superoxide anion; ROS: Reactive oxygen species; FW: Fresh weight, EcLOX: Eleusine coracana lipoxygenase, PRO: Proline

INTRODUCTION

Abiotic stress like water deficit, salinity, heavy metals and high temperature affect several physiological processes of plants from among them; drought is the most important limiting factor for crop production and it is became an increasingly severe problem in many regions of the world [1, 2]. Plants maintain cellular homeostasis under abiotic stress by adjusting their metabolic systems. The early events of plant adaptation to the environmental stress involve stress-signal perception and transduction leading to the expression of stress responsive genes and activation of various physiological and metabolic responses [3]. The crop plants can respond and adapt to water stress by altering in their cellular metabolism and evoking various defense mechanisms [4]. The production of cytotoxic reactive oxygen species (ROS) like super oxide (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH-) can seriously disrupt normal metabolism during stress through chlorophyll loss, membrane lipid peroxidation, protein carbonylation and inactivating the –SH containing enzymes.

A highly efficient antioxidant defense system is present in the plant cells for ROS detoxification which includes either the non-enzymatic constituents like tocopherols, anthocyanin, flavonoids, carotenoids or enzymatic constituents, like SOD, CAT, GPX, ascorbate peroxidase (APX) or glutathione reductase (GR). The SOD converts superoxide to H_2O_2 and O_2; H_2O_2 is then scavenged by CAT and a variety of peroxidases by the oxidation of co-substrates such as phenolics or other antioxidants.

Lipoxygenases (LOXs; EC 1.13.11.12) are a family of dioxygenases that catalyze regio- and stereo- specific dioxygenation of polyunsaturated fatty acids with one or several cis, cis-1, 4-pentadiene units to form hydroperoxy fatty acids [5]. The enzyme is widely distributed in a large variety of organisms such as mammals, fish, plants, fungi, and yeast [6], indicating the biological importance of these enzymes. LOX isoenzymes are nearly ubiquitous in the plant kingdom and are involved in many physiological processes such as flowering [7], seed germination [8], pigment bleaching [9], formation of flavour and aroma in plant products [10] plant growth and development [11]. Isoenzymes of LOX difference in their substrate specificity and pH optimum are located in cytosol [12], microsomes [13], plasma membrane [14] and oil bodies [15]. LOXs producing hydroperoxy fatty acids, which are highly reactive compounds that are toxic and initiate lipid peroxidation and cause damage to cell components. LOX-derived fatty acid hydroperoxides can be further metabolized into volatile aldehydes and jasmonates and plays an important role in plant physiology [16].

Figner millet (Eleusine coracana (L) Gaertn) is one of the ancient millets in India (2300 BC), of all the cereals and has highest amount of calcium (344 mg %) and potassium (408 mg %). It has higher dietary fiber, minerals, and sulfur containing amino acids compared to white rice, current major staple food of Africa and Asia, which is widely grown in semi-arid and arid tropics. Incidentally, the arid and semi-arid zones that are primarily affected by water deficit, have traditionally contributed around 40 per cent of the total production of all categories of food grains. Finger millet survives under severe water-deficit and osmotic stress and shows remarkable recovery on alleviation of stress [17]. Although, this crop is adapted to resist severe drought [17], little is known about its mechanisms of osmotic adjustment and ability to repair the damage caused by drought-induced oxidative stress. Further, very little is known, about the link between drought stress, LOX activity, antioxidant enzymes, PRO and MDA levels, response in the millets. The aim of this study is to investigate the mechanism of Lipoxygenase and other antioxidant enzymes response in millet plants (Sri Chaitanya VR-847, drought tolerant) under drought stress.

MATERIAL AND METHODS

Plant materials and drought treatments

Finger millet seeds were obtained from Regional Agricultural Research Station, Vizaya Nagaram, Andhra Pradesh, India. Finger millet seeds (Sri Chaitanya VR-847) are surface sterilized with 0.1 %(w/v) $Hgcl_2$ for 10min, and they are imbibed in deionized water for 10–12 h and allowed to germinate at 27^0C in dark, after that they are transferred to plastic pots and exposed to photoperiod for 12hrs, 30days at 27 ^{0}C for plant growth. Then plants were divided in to control and test groups (Drought stress group) for further experiments. Control plants were supplemented with water and test samples were withhold water supplement for 7days. Leaves were collected at every 24hours intervals from both plants for investigation.

Estimation of chlorophyll content

Chlorophyll content from both control and test plant samples were estimated by using the method of Arnon, 1949 [18]. About 1gm of leaf sample was homogenized in a pre-cooled mortar and pestle using 80 %(v/v) acetone. The extract was centrifuged at 3000rpm for 15min and made up to 25ml with 80 %(v/v) acetone. The clear solution was transferred to a cuvette and optical density was measured at 645nm and 663nm against blank (80 %(v/v) acetone).

Lipid peroxidation

The rates of lipid peroxidation levels in control and test plant leaves were determined by the method of Heath and Packer, 1968 [19] by measuring amount of its ability to inhibit the photochemical Malondialdehyde (MDA) formed by the thiobarbituric reduction. Leaves were grounded with mortar and pestle in 1% TCA and centrifuged at 10000 rpm for 10 mins at room temperature. To 1.0ml of supernatant add 4ml of 20% TBA-TCA solution. The mixture was heated at 95^0C for 30min. Absorbance was measured at532nm and corrected for unspecific turbidity by subtracting the value at 600nm. The blank contained 20%TBA-TCA solution. MDA content was calculated using an extinction coefficient of 155 mM $^{-}$1 cm^{-}1and the results were expressed as µmol MDA g $^{-}$1FW.

Hydrogen peroxide quantification

Hydrogen peroxide level was determined by the method of Velikova, et al., 2000 [20]1gm of leaf samples are homogenized by adding 0.5ml of 0.1 %(w/v) TCA and centrifuged at 15000rpm for 15min at 4^0C. Then 0.5ml of each supernatant was added to 0.5 ml of 10mM phosphate buffer, pH 7.0 and 1 ml of 1M potassium iodide (KI). The absorbances of the supernatants were measured at 390 nm. H_2O_2 is quantified taking in to account a calibration curve using solutions with known H_2O_2 concentrations and results are expressed as µmol H_2O_2 g^{-} 1 FW.

Proline level

Determination of free proline content was done according to Bates et al., 1973 [21]. Leaf samples (0.5 g) from each group were homogenized in 3% (w/v) sulphosalycylic acid and homogenate filtered through filter paper.

After addition of acid ninhydrin and glacial acetic acid, , the resultant mixture was heated for 1hr at 100 ^{0}C water bath water bath. Reaction was then stopped by using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as µmol proline g−1.

Lipoxygenase enzyme activity

The leaves of the finger millet were blended into a fine powder and was suspended in50mM phosphate buffer at pH 6.4 containing 1mM Phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged for 10 min at 12000g/10min at 4 ^{0}C. The resulting supernatant was subsequently assayed for lipoxygenase activity. Lipoxygenase activity was measured using Shimadzu UV-VIS spectrophotometer, at 25 ^{0}C by monitoring the increase in absorbance over a period of time at 234 nm [22]. The typical reaction mixture contains 2.8 ml of 50mM sodium phosphate buffer pH 6.4, the appropriate volume of the enzyme (10-100µl) and the reaction was initiated by addition of substrate to the reaction mixture and maintained to have 250µM for linoleic acid in the total volume. One unit of enzyme activity is defined as the amount of micro moles of hydroperoxide formed per minute. The lipoxygenase activity is calculated by using the following formula.

$$\text{Enzyme activity} = \frac{\Delta difference \times Total\ volume\ of\ the\ reaction\ mixture}{e \times volume\ of\ the\ enzyme}$$

$$e = 0.00275$$

Expression analysis of Lipoxygenase by Semiquantitavite RT-PCR

Finger millet lipoxygenase gene expression of control and test plants were analyzed by semiquantitavite RT-PCR. Total RNAs was extracted from100mg of control and test plant leaves for the analysis of lipoxygenase gene expression in response to the drought condition by using semi quantitative RT-PCR.2µg of total RNA was reverse transcribed in to cDNA by using Prime Script 1st strand cDNA synthesis kit (TAKARA, Japan) following manufactures instructions and 1/10 volume of cDNA was used as template in PCR reaction. The Lox gene specific primers FMF: 5'-CAGGCG TGGTGGAAGGAG -3', FMR: 5'-GGACATCACGCCCGAGT C -3' were used in PCR amplification reactions under the following conditions mentioned: Initial denatured at 94 °C for 3min, followed by 25 cycles of 30 s at 94 °C, 30 s at 50 °C, 30sec at 72 °C, and final extension at 72 °C for 5 min. For an endogenous control actin was amplified using actin specific primers: Actin-F: 5'-GCCCTCCTCCTCCTCCTC-3' and Actin-R: 5'-GATTATGGAGCGGGTGATGC -3'. The PCR conditions for actin amplification are initial denaturation at 94 °C for 3 min, followed by 25 cycles of amplification (94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1min) and final extension at 72 °C for 5 min. The amplificons were resolved on 1% agarose gel.

Enzyme extractions and antioxidant bioassay

For protein and enzyme extractions, 1 g of leaf samples were homogenized with 50mM sodium phosphate buffer (pH 6.4) containing 1mM EDTA·Na2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). The whole extraction procedure was carried out at 4^{0}C.Homogenates were then centrifuged at 4^{0}C for 30 min at 15000rpm and supernatants were used for determination of enzyme activity. Protein concentration was determined by method of Bradford, 1976 [23] using bovine serum albumin as a standard.

Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Cakmak, I. and H. Marschner, 1992 [24] by measuring the initial rate of disappearance of H_2O_2. The reaction mixture contained 0.1ml of 0.05MNa–phosphate buffer (pH 7.6), 0.1ml of 0.1mM EDTA, 0.1ml of 100mM $H_2O_2$and 0.7ml of enzyme aliquot. The decrease in H_2O_2 was measured as a decline in optical density at 240 nm, and activity was calculated as µmol H_2O_2 consumed per minute.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed spectrophotometrically by the method of Beauchamp, C. and I. Fridovich, 1971 [25], as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm. The reaction mixture contained 33µM NBT, 10mM L-methionine, 0.66mM EDTA·Na2, and 0.0033mM riboflavin in 0.05M Na–phosphate buffer (pH 7.8). The reaction was initiated by final addition of 0.044 g/mL riboflavin and the mixtures were shaken and waited for 10 min under 300molm−2 s−1 irradiance at room temperature. The reaction mixture with out enzyme developed maximum colour due to maximum reduction of NBT.

A non-irradiated reaction mixture did not develop colour and served as control. The reduction of NBT was inversely proportional to SOD activity. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photo reduction.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following method of Nakano,Y and K. Asada, 1981 [26]. The reaction mixture contained 0.05M Na–phosphate buffer (pH 7), 0.5mM ascorbate, 0.1mM EDTA·Na2, 1.2mM H2O2, and 0.1mL enzyme extract in a final assay volume of 1 mL. Ascorbate oxidation was measured at 290 nm. The concentration of oxidized ascorbate was calculated by using extinction coefficient (ε = 2.8mM−1 cm−1). One unit of APX was defined as 1 mmol mL−1 ascorbate oxidized per minute.

Glutathione reductase (GR; EC 1.6.4.2) activity was measured according to Foyer, C.H. and B. Halliwell, 1976 [27], the assay medium contained 0.025mM Na–phosphate buffer (pH 7.8), 0.5mM GSSG, and 0.12mM NADPH.Na4, and 0.1mL enzyme of extract in a final assay volume of 1 mL. NADPH oxidation was determined at 340 nm. Activity was calculated using the extinction coefficient (ε =6.2mM−1 cm−1) for GSSG. One unit of GR was defined as 1 µmol mL−1 GSSG reduced per minute.

Statistical analysis.

In general, mean values are examined statistically by using one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ followed by Turkey–Kramer multiple comparison tests.

RESULTS AND DISCUSSION

Effect of drought on chlorophyll content, lipid peroxidation and H_2O_2 levels.

The drought stress was established by withholding water supplement to the finger millets seedlings. The change in water supply led to pronounced changes in chlorophyll content, lipid peroxides and H_2O_2 levels in millets (Figure.1). Significant decrease was noticed in total chlorophyll content in test samples in response to drought. However, in control samples no reduction was observed (Figure 1A). Drought stress reduces the availability of CO_2 in leaves and inhibits the carbon fixation and generation of ROS which leads to oxidative stress.

Lipid peroxidation levels in control and test samples were given (Figure 1B) and levels were measured as the content of MDA in control and test samples. A small increase in lipid peroxidation level became apparent after 3 days, which was statistically insignificant for control, in contrast 1.5 fold significant increments was observed on day 5 and 6 in test sample (Figure 1B). Detection of degradation PUFA products such as MDA is general concept for considering the degree of stress effect in response to different environmental stress in plants [28]. These electrophile species MDA derived from fatty acid degradation shows regulatory activity on plant defense gene expression [29]. In this study, the MDA levels were increased in response to drought in test samples and were significant.

Hydrogen peroxide (H_2O_2) is an important signaling molecule and any increase in H_2O_2 has severe consequences for the affected cell and is a well-known reactive oxygen species. The H_2O_2 level in control and test sample leaves were determined and calculated on the basis of standard curve (Figure 1C). Control samples shows steady state levels of H_2O_2 when compared with the test samples. In contrast, with increasing days of drought condition, a significant enhancement (2 fold) in the H_2O_2 content was observed in test sample leaves on every sampled day. A change in H_2O_2 content is a good indicator of the status of ROS scavenging capacity of plants under oxidative stress. The efficiency of such scavenging is monitored by estimating the H_2O_2 content in leaves upon exposure to drought conditions. The excess oxygen in the plant is then used in the formation of ROS and levels of ROS production increased when a plant is under abiotic stress [30]. During drought stress, the H_2O_2 generation was showed marked difference (2 fold) with control in test samples.

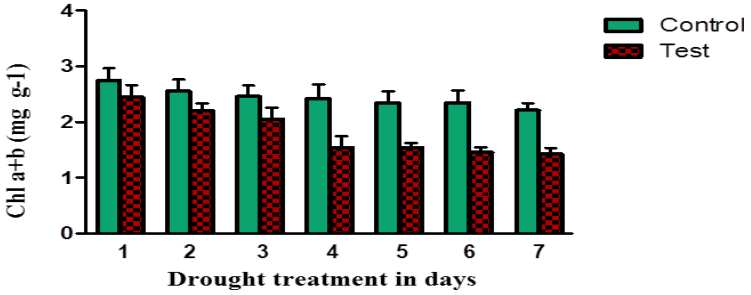


Fig 1A: Effect of drought stress on total chlorophyll content in finger millet leaves from day 1-7, and chlorophyll content expressed in mg g^{-1}

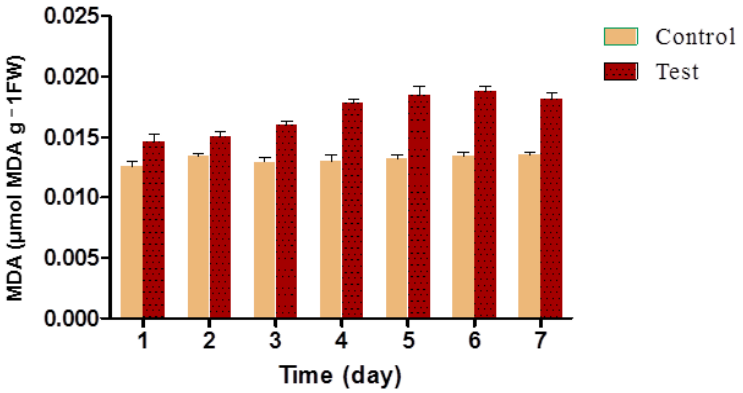


Fig 1B. The effect of drought stress on MDA levels in finger millet leaves from day 1-7, MDA levels in lipid peroxidation expressed in µ mol MDA g-^{1} FW.

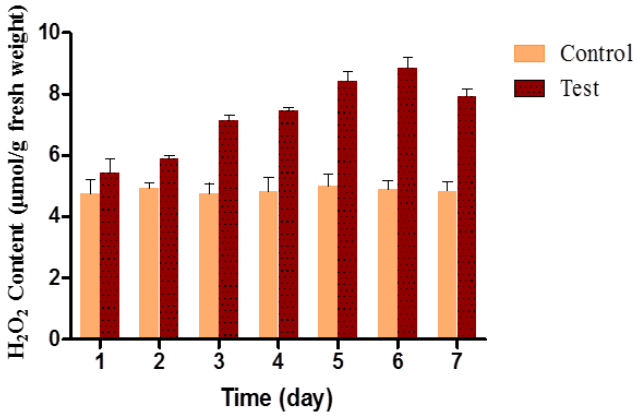


Fig 1C. The effect of drought stress on H_2O_2 content in finger millet leaves from day 1-7, H_2O_2 levels expressed in µ mol g-^{1} FW.

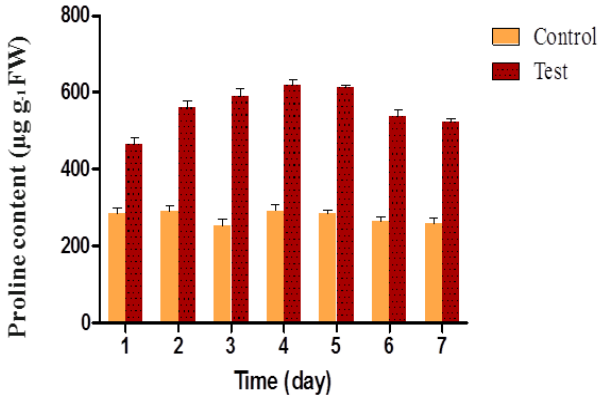


Fig 2. The effect of drought stress on proline content in finger millet leaves from day 1-7, proline levels expressed in µg g-^{1} FW.

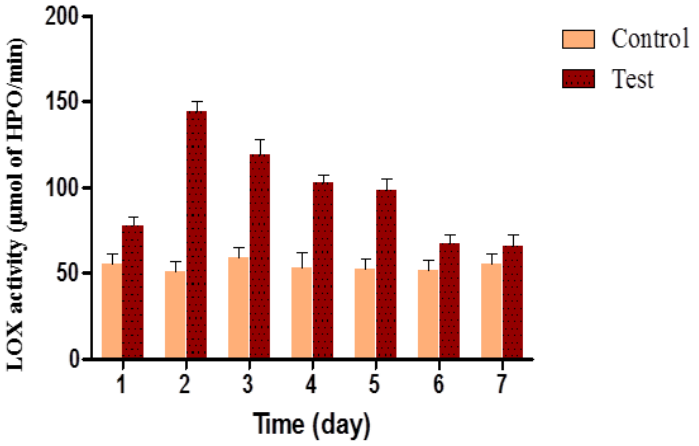


Fig 3A. Lipoxygenase enzyme activity from finger millet leaves. A crude enzyme is isolated from test and control samples on day 1-7. One unit of enzyme activity is defined as the amount of µMoles of hydroperoxides formed per minute.

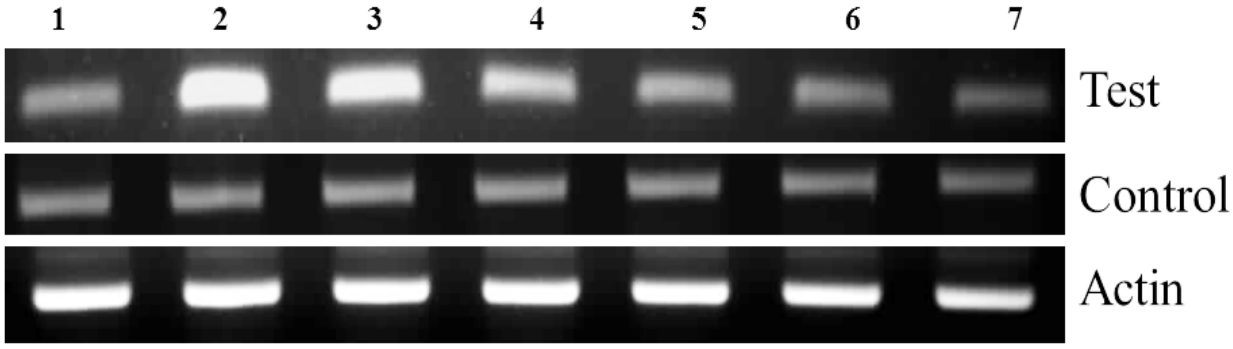
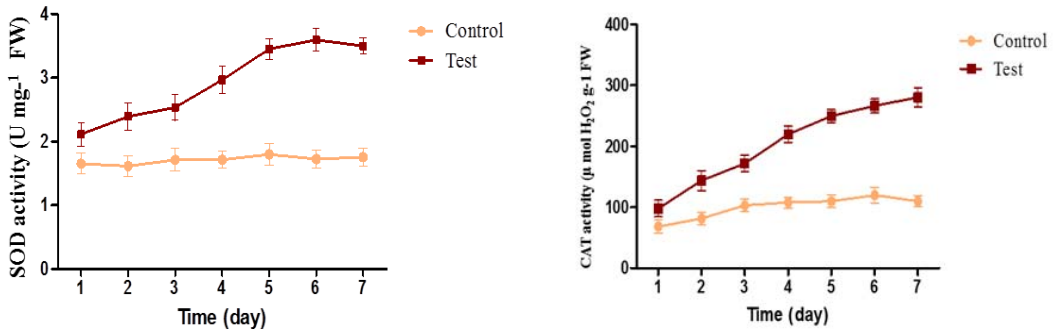
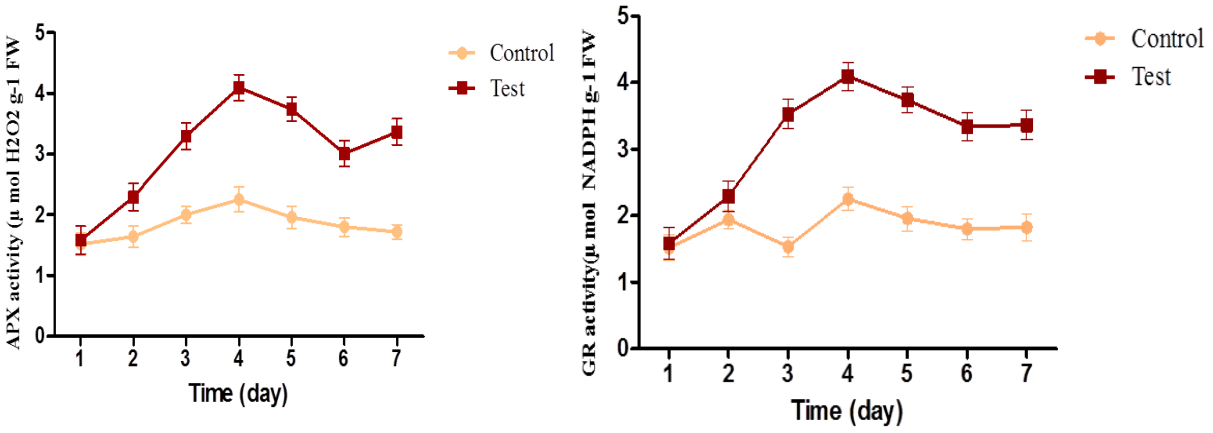


Fig 3B. Expression levels of EcLOX gene under drought condition. Total RNA isolated from finger millet test and control leaves at the interval of every 24 hour utilized to measure the levels of LOX gene expression using gene specific primers. Actin Specific primers were used to amplify actin gene as an additional control in semi-quantitative RT-PCR.



A) **B)**



C) **D)**

Fig 4. Effect of drought stress on antioxidative enzymes in finger millet leaves response to drought from day 1-7
A) Activity of Super oxide dismutase (SOD), expressed in Unit mg^{-1} protein
B) Activity of Catalase (CAT), expressed in µ mol H_2O_2 g^{-1} protein
C) Activity of Ascorbate peroxidase (APX), µ mol H_2O_2 g^{-1} protein.
D) Activity of Glutathione reductase (GR), µ mol NADPH g^{-1} protein [Data is means ± SD (n=3) and error bars are significantly different at $p<0.05$].

Table-1: Primer sequence

S.No	Primer Name	Forward Primer
1	FM F	5'-CAGGCG TGGTGGAAGGAG-3'
2.	Actin F	5'-GCCCTCCTCCTCCTCCTC-3'
S.No	**Primer Name**	**Reverse Primer**
1	FM R	5'-GGACATCACGCCCGAGTC -3'
2.	Actin R	5'-GATTATGGAGCGGGTGATGC-3'

Proline levels

It is well established that drought stress results in an increase of proline biosynthesis rate. The proline content was measured from control and test leaves of finger millet by using a standard curve of L-Proline (Figure 2). Obtained results revealed that, proline content increased more than 3 fold upon exposure to drought condition in test samples on comparison with control samples. Accumulation of free proline is a typical response to drought stress. In many plants accumulation of high quantity proline was reported [31]. Proline is an important osmoprotectant and is known to be involved in alleviating cytosolic acidosis associated with several stresses [32] .

Lipoxygenase enzyme activity and expression analysis

In plant, lipoxygenases play a key role in many physiological processes and biotic/ abiotic stress responses. It is identified marked increment (2fold) in the lipoxygenase enzyme activity in response to drought exposure, where as in control the LOX activity was remained more or less constant levels in finger millet leaves (Figure 3A).

A marked up-regulation of lipoxygenase gene expression was also detected on day 3, and gene levels were declined gradually thereafter in response to drought stress (Figur 3B). In drought stress plant organs are affected, particularly leaves are most affected by water deficit. In our studies Lox shows marked increase in the leaves of finger millet. It is believed that, during stress, oxygenases play an active role in PUFA oxidations. Jasmonates and its derivatives originate from the lipid peroxidation via lipoxygenase (LOX) pathway play an important role in biotic and abiotic stress [33]. The gradual increase of LOX activity during the progression of water deficit suggested a strict relationship of this enzyme with drought-stress conditions [34]. The results obtained in this investigation may be important for complete understanding of the mechanism of lipoxygenases in the millet crops in arid and semi-arid regions.

Effect of drought on antioxidant enzymes

The levels of ROS are regulated in the biological system by the action of antioxidant enzymes [35]. SOD is the first defense enzyme that catalysed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2, which is further metabolized by CAT or GPX reactions [36]. In this study SOD activity increased for test samples to 1.5 fold when compared with control plants (Figure 4A). The reactive oxygen species (ROS) generated in drought stress is highly reactive and causes denaturation, mutagenesis or lipid peroxidation by reacting with macromolecules and resulting in metabolic disturbances [37]. SOD action leads to the production of H_2O_2, a highly toxic molecule leads to living cell, which needs to be eliminated from plant cells in subsequent reactions.

Catalase (CAT) is an enzyme responsible for the degradation of hydrogen peroxide present in plant leaves. Catalase (CAT) activity was increased to 0.8 fold in test samples when compared to controls in millets for drought response (Figure 4B).

A significant increase in Ascorbate peroxidase (APX) specific activity was also recorded in drought stressed plants as compared to the control plants (Figure 4C), a maximum increase of 1.5 folds in the APX activity was observed in drought exposed samples. The APX another metabolic enzyme detoxifies H_2O_2 plays a crucial role in management of ROS during oxidative stress in plants[38]. In this study, the obtained results showed that enzymatic activity of SOD, CAT and APX increased significantly in test sample leaves under drought stress conditions over their control samples.

The Glutathione reductase (GR) activity was examined and 0.9 folds increment was observed in test sample plants over their control samples in drought response (Figure 4D). Glutathione reductase (GR) is an important cellular antioxidant enzyme, also known as GSR or GR which reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, [39]. In a wide range of plant species, increase in GR activity was observed under different abiotic stress conditions [40]. Furthermore it is also showed that, a noticeable raise in Lox activity under drought stress in millets. Our results support the hypothesis that lipoxygenases along with coordination of antioxidative enzymes plays central role in the survival of plant under drought stress.

CONCLUSION

In conclusion, the results show that drought stress is accompanied by oxidative stress in finger millet leaves. The present findings indicate that finger millet plant leaves responded to water deficit stress by enhancing their antioxidative capacity. Antioxidative enzymes such as CAT, SOD, APX, GR plays an important role in the overall oxidative stress tolerance potential in finger millet. Comparatively higher specific activity of APX under drought stress, as compared to GR and CAT, further substantiate the role of APX to be of critical importance for the detoxification of stress induced H_2O_2. For the first time our experiments indicate that the lipoxygenases play an important regulatory role in drought tolerance along with antioxidant enzymes in finger millet crop. Gradual increase of LOX activity during the progression of water deficit suggests a strict relationship of this enzyme with drought-stress conditions. Complete knowledge at molecular level on lipoxygenase is essential how they regulate and cross talk in response to drought in millets.

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REFERENCES

[1]. RoyChoudhury, A., C. Roy, and D.N. Sengupta, 2007. Transgenic tobacco plants overexpressing the heterologous lea gene Rab16A from rice during high salt and water deficit display enhanced tolerance to salinity stress. Plant Cell Rep. 26(10): p. 1839-59.

[2]. Passioura, J., 2007. The drought environment: physical, biological and agricultural perspectives. J Exp Bot, 58(2): p. 113-7.

[3]. Bray, E.A., 1993. Molecular Responses to Water Deficit. Plant Physiol, 103(4): p. 1035-1040.

[4]. Nguyen TTT, Klueva N, Chamareck V, Aarti A, Magpantay G, Millena ACM, Pathan MS, Nguyen HT (2004). Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Mol Genet Genomics, 272(1): p. 35-46.

[5]. Andreou, A. and I. Feussner, 2009. Lipoxygenases - Structure and reaction mechanism. Phytochemistry, 70(13-14): p. 1504-10.

[6]. Joo, Y.C. and D.K. Oh, 2012. Lipoxygenases: potential starting biocatalysts for the synthesis of signaling compounds. Biotechnol Adv, 30(6): p. 1524-32.
[7]. Ye Z, Rodriguez R, Tran A, Hoang H, de los Santos D, Brown S, Vellanoweth L (2000). The developmental transition to flowering represses ascorbate peroxidase activity and induces enzymatic lipid peroxidation in leaf tissue in Arabidopsis thaliana. Plant Sci, 158(1-2): p. 115-127.
[8]. Suzuki, Y. and U. Matsukura, 1997. Lipoxygenase activity in maturing and germinating rice seeds with and without lipoxygenase-3 in mature seeds. Plant science, 125(2): p. 119-126.
[9]. . Pastore D, Trono D, Padalino L, Simone S, Valenti D, Di Fonzo N, Passerella S (2000). Inhibition by α-tocopherol and L-ascorbate of linoleate hydroperoxidation and β-carotene bleaching activities in durum wheat semolina. Journal of Cereal Science, 31(1): p. 41-54.
[10]. Williams M, Salas JJ, Sanchez J, Harwood JL (2000). Lipoxygenase pathway in olive callus cultures *(Olea europaea)*. Phytochemistry, 53(1): p. 13-9.
[11]. Hildebrand, D.F., R.T. Versluys, and G.B. Collins, 1991. Changes in lipoxygenase isozyme levels during soybean embryo development. Plant science, 75(1): p. 1-8.
[12]. Siedow, J.N., 1991. Plant lipoxygenase: structure and function. Annual review of plant biology, 42(1): p. 145-188.
[13]. Feussner, I. and H. Kindl, 1994. Particulate and soluble lipoxygenase isoenzymes. Planta, 194(1): p. 22-28.
[14]. Macri F, Braidot E, Petrusa E, Vianello A., 1994. Lipoxygenase activity associated to isolated soybean plasma membranes. Biochim Biophys Acta, 1215(1-2): p. 109-14.
[15]. Rodrıguez-Rosales MP, Kerkeb L, Ferrol N, Donaire JP (1998). Lipoxygenase activity and lipid composition of cotyledons and oil bodies of two sunflower hybrids. Plant Physiology and Biochemistry, 36(4): p. 285-291.
[16]. Mosblech, A., I. Feussner, and I. Heilmann, 2009. Oxylipins: structurally diverse metabolites from fatty acid oxidation. Plant Physiol Biochem, 47(6): p. 511-7.
[17]. Govind G, Harshavardhan V, Patricia JK, Dhanalakshmi R, Senthil Kumar M. (2009). Identification and functional validation of a unique set of drought induced genes preferentially expressed in response to gradual water stress in peanut. Mol Genet Genomics, 281(6): p. 591-605.
[18]. Arnon, D.I., 1949. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. Plant Physiol, 24(1): p. 1-15.
[19]. Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys, 125(1): p. 189-98.
[20]. Velikova, V., I. Yordanov, and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science, 151(1): p. 59-66.
[21]. Bates, L., R. Waldren, and I. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant and soil, 39(1): p. 205-207.
[22]. Reddanna, P., Whelan, J., Maddipati, K. R., Reddy, C. C., 1990. Purification of arachidonate 5-lipoxygenase from potato tubers. Methods Enzymol, 187: p. 268-77.
[23]. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: p. 248-54.
[24]. Cakmak, I. and H. Marschner, 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol, 98(4): p. 1222-1227.
[25]. Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem, 44(1): p. 276-287.
[26]. Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology, 22(5): p. 867-880.
[27]. Foyer, C.H. and B. Halliwell, 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta, 133(1): p. 21-5.
[28]. Jain, M., Mathur, G., Koul, S., Sarin, N.B., 2001. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut *(Arachis hypogaea L.)*. Plant Cell Rep, 20(5): p. 463-468.
[29]. Almeras, E., Stolz S, Vollenweider S, Reymond P, Mene-Saffrane L, Farmer EE 2003. Reactive electrophile species activate defense gene expression in Arabidopsis. Plant J, 34(2): p. 205-16.
[30]. Xiong, L. and J.K. Zhu, 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant, Cell & Environment, 25(2): p. 131-139.
[31]. Mansour, M., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. Biologia Plantarum, 43(4): p. 491-500.

[32]. Kurkdjian, A. and J. Guern, 1989. Intracellular pH: measurement and importance in cell activity. Annual review of plant biology, 40(1): p. 271-303.
[33]. Wasternack, C., 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Annals of botany, 100(4): p. 681-697.
[34]. Adriano Sofoa, Bartolomeo Dichioa, Cristos Xiloyannisa and Andrea Masiab 2003. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. Physiologia Plantarum, 121(1): p. 58-65.
[35]. Hodges, D.M., 2003. Postharvest oxidative stress in horticultural crops.
[36]. Fridovich, I., 1995. Superoxide radical and superoxide dismutases. Annual review of biochemistry, 64(1): p. 97-112.
[37]. Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in plant science, 7(9): p. 405-410.
[38]. Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology, 49(1): p. 249-279.
[39]. Meister, A., 1988. Glutathione metabolism and its selective modification. J Biol Chem, 263(33): p. 17205-17208.
[40]. Israr, M., S. Sahi, and J. Jain, 2006. Cadmium accumulation and antioxidative responses in the Sesbania drummondii callus. Archives of environmental contamination and toxicology, 50(1): p. 121-127.