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INFLUENCE OF DROUGHT STRESS AND MECHANICAL WOUNDING ON LIPOXYGENASE ACTIVITY AND BIOCHEMICAL CHANGES IN HORSE GRAM (*Macrotyloma uniflorum*).

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ABSTRACT: This work deals with the comparative analysis of the lipoxygenase activity and a few biochemical changes that were generated to reactive oxygen species produced in excess during abiotic stress conditions as drought and mechanical wounding in Horse gram (*Macrotyloma uniflorum*) leaf tissue. Plants exposed to different types of stress conditions like drought, salinity, cold, mechanical damage etc., shows retardation in growth and productivity. Such negative impacts on the plant growth were caused by the reactive oxygen species generated during the stress conditions, which alter the biochemical parameters like H_2O_2, Proline, Malondialdehyde content etc. Lipoxygenase enzyme produces the reactive oxygen species which may be lethal to plant if present in excess. During drought, sparse elevations in lipoxygenase activity and biochemical entities were observed during initial days in test samples in comparison with control and a gradual increment occurs in further sampling days. In contrast to drought stress, initial high elevations in lipoxygenase activity and biochemical entities was observed in test samples during mechanical wounding in comparison with control and the variations reduced gradually on further sampling days which concludes a slow and higher changes in lipoxygenase activity and biochemical entities during drought and a quick and comparatively lesser changes during mechanical wounding.
Key Words: Lipoxygenase, Reactive oxygen species, Drought, Mechanical wounding.

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Abbreviation: LOX-Lipoxygenase; ROS-Reactive oxygen species; H_2O_2-Hydrogen peroxide; MDA-Malondialdehyde; OD-Optical density; TBA-Thiobarbituric acid; TCA-Trichloroacetic acid.

INTRODUCTION

Horse gram (*Macrotyloma uniflorum*), is a member of *Fabaceae* family. *Fabaceae* family gains importance as the second most important crops to human beings [1]. It is having its importance as a cheap source of iron and protein and is known as poor man's pulse. Horse gram is also used for livestock production [2]. The plant of horse gram is known to show antioxidant, astringent and diuretic action useful in the treatment of hemorrhoids, diarrhea, hemorrhage and leucorrhea accounts for its medicinal properties.

Horse gram is a drought resistant plant grown in the tropic and subtropics areas under dry land cultivation [3, 4]. Drought occurs frequently in dry lands as the average rain fall will be low. The crops grown here were under stressed conditions as the moisture content in the soil does not meet the requirements of the plants over a longer period of time. The stressed condition of the plant leads to the retard growth and productivity. Stress prone plants produce ROS in excess, that are lethal to plant and their effects have to be nullified for its survival.

Over-accumulation of ROS occurs either because of their over-production [5]or owing to dysfunctioning of antioxidative defense becoming unable to scavenge them fully [6, 7].Many enzymatic and non-enzymatic systems were involved in scavenging the ROS produced in excess. Our present study carried out is to know the LOX activity variations along with the biochemical changes during abiotic stress conditions like drought and mechanical wounding.Plants display a wide variety of physiological and biochemical responses at cellular and whole-organism levels towards drought stress and mechanical wounding conditions to get rid of the effect caused by the reactive oxygen species, thus making stress management a complex phenomenon [8].

LOX enzyme gain its importance in stress studies as it mediates the formation of singlet oxygen or a superoxide, which are potential ROS. LOX are the non-heme, iron dependent, regio- and stereo-specific enzymes which catalyzes the formation of hydroperoxides through the dioxygenation of polyunsaturated fatty acids in (*1Z, 4Z*)-penta diene system [9]. They are widely distributed in living organisms ranging from plants, animals, fungi and some bacteria. The LOX catalyzed hydroperoxides, otherwise called oxylipins, produced in LOX pathways, have vital roles to play in the physiology of the plant. The production of the oxylipins depends on the availability of the substrate to the enzyme and the substrate availability is greatly altered during biotic, abiotic stress conditions and during the development of a plant, which intern changes the oxylipins profile as per the need of the plant [10]. LOX gene expression modulation in soybean during drought, mechanical wounding and methyl jasmonates has been reported [11]. Enhanced LOX activity was observed regarding to LOX-1 and LOX-2 during osmotic stress in soybeanplant [12]. Mechanical wounding also induces the LOX in mature leaves was reported in common bean suggests its necessity during stressed condition of the plant [13].

The biochemical entities under study have deleterious effects on plants if present in excess. $H_2O_2$is the most stable, active, toxic and destructive reactive oxygen species causing deleterious effect in plants [14]. Lipid peroxidation produces MDA content, acts as an indicator of oxidative damage. It occurs due to direct attack of ROS on lipids, proteins and their destruction [15]. Proline acts as stress related signal molecules [16]. It also plays a role as osmolytic biomolecule not only stabilizing the proteins but also maintain cell membrane structure. Proline acts as free radical scavengers helps in maintaining cellular homeostasis.

MATERIALS AND METHODS

Plant Material and growth conditions

Horse gram (*Macrotyloma uniflorum*) seeds were obtained from agricultural Farm of Andhra Pradesh Agricultural University, Rekulakunta, Anantapur, Andhra Pradesh, India. These seeds were selected for uniformity in size and surface sterilized with 0.1%w/v mercuric chloride ($HgCl_2$) for 5 min followed by thorough washing with sterile double distilled water for 4-5 times. The sterilized seeds were sown in pots of uniform size with red soil and cow dung (natural manure) in a proportion of 2:1. Plants were grown in a greenhouse and watered regularly once in day for 25 days. After 25 days, the plants were subjected to drought stress by dewatering the plants, mechanical stress by wounding the plant leaf with a sterile surgical blade. The effects of these stress on biochemical characters was studied and analyzed.

Growth Study

To study the effect of drought on biochemical characters few parameters were studied on 0 day (2 hours after inducing the stress),1^{st} day,3^{rd} day,5^{th} day,7^{th} day (wilting starts) 9^{th} day and 10^{th} day (complete wilting of the plant). A similar study of biochemical parameters was carried out from day 0 to day 10 in mechanical wounding stress with the sampling intervals followed similar to drought stress. The Different parameters studied were the LOX enzyme activity, H_2O_2 peroxidation, MDA content and proline assay.

Biochemical Analysis

LOX activity

LOX enzyme activity was determined spectroscopically with a modified protocol mentioned by Reddanna *et al*[17]. It was carried out with a crude enzyme obtained from Horse gram leaves of stress and control plants, with LOX substrate solution, linoleic acid and the formation of conjugate hydroperoxydienes was measured at 234 nm using a double beam spectrophotometer (Shimadzu, 2101 PC) and a 1 cm path-length cuvette. One unit of LOX activity was defined as an increase in absorbance at 234 nm by 0.001 per minute per mg of protein under assay conditions [18].

H_2O_2 quantification

For H_2O_2 quantification, 100 mg of leaf tissue was weighted and ground with 1 ml of 0.1% TCA (W/V). The homogenate was centrifuged at 15,000x g for 15 minutes at 4^0C. An aliquot of 0.5 ml is added to 0.5 ml of 10 mM Phosphate buffer and 1.0 ml of 1M Potassium iodide and absorbance was measured at 390 nm. A calibration graph was drawn with known H_2O_2 concentrations and the test H_2O_2 is quantified with reference to the graph and expressed as H_2O_2 g^{-1} fresh weight [19].

Lipid peroxidation

Lipid Peroxidation was carried out using the following procedure suggested by Heath &packer [20].100 mg of leaf tissue was homogenized by adding 0.5 ml 0.1% TCA and centrifuged the homogenate for 10 minutes at 15000 x g at 4^0C. 0.5 ml of the collected supernatant was mixed with 1.5 ml of 0.5% TBA diluted in TCA and incubated for 25 minutes at 95^0C in a water bath. The reaction was terminated by incubating on ice for 10 min and absorbance was measured at 532nm and 600 nm. OD values are subtracted from the MDA-TBA complex values at 532 nm and MDA concentration is calculated using the Lambert-Beer law with an extinction co efficient ϵ^M=155mM-1cm^{-1} .Results are presented µM of MDA g $^{-1}$FW.

Proline assay

Proline assay carried out by following the method suggested by Bates et al [21]. The frozen plant material is homogenized in 3% aqueous sulfosalicylic acid (0.01g/ 0.5ml) and the residue is removed by centrifugation at 12,000 x g for 10 min.1 ml of the homogenized tissue reacts with 1 ml acid-ninhydrin and 1 ml glacial acetic acid in a test tube for 1 hour at 100^0C and the reaction is terminated in an ice bath. The reaction mixture is extracted with 2 ml toluene, mixed vigorously and left at room temperature for 30 min until separation of the two phases. The chromophore-containing toluene (1 ml, upper phase) is warmed to room temperature and its OD is measured at 520 nm using toluene for a blank. The proline concentration is determined from a standard curve using D-proline. Statistical analysis was carried out using MATLAB 7.0.

RESULTS AND DISCUSSION

LOX activity

LOX activity studies in drought stress, we observed test sample (271.63 µM hydroperoxy dienes / g. f.w.t) has slightly higher LOX activity by 0.04 folds (4%) over control (260.72 µM hydroperoxy dienes / g.f.w.t) on day 0 and later on showed 0.07 folds (7 %) hike on day 1 in test sample (322.54 µM Hydroperoxy dienes / g .f.w.t) in comparison with control sample (300.72 µM hydroperoxy dienes / g .f.w.t). On day 3, the test sample showed a similar hike by 0.07 folds (7%) in test sample (369.81 µM Hydroperoxy dienes / g.f.w.t) in comparison with the control (344.36 µM Hydroperoxy dienes / g .f.w.t). 5th Day test sample showed (556 µM hydroperoxy dienes / g .f.w.t) 0.16 folds (16%) hike over the control (478.9 µM hydroperoxy dienes / g.f.w.t), marking a clear elevation in the activity in response to stress. On 7th day, the day when the plant leaf started to wilt test sample (584 µM hydroperoxy dienes / g .f.w.t) showed a maximal hike of 0.57 folds (57%) in comparison with control (369.81 µM hydroperoxy dienes / g.f.w.t) .On day 9, the hike in test sample (339.63 µM hydroperoxy dienes / g.f.w.t) was up to 0.01 folds (10%) in comparison with control (308 µM hydroperoxy dienes / g.f.w.t) and a similar change as of day 9 was observed in LOX activity on day 10, where in the test sample (322.9 µM hydroperoxy dienes / g.f.w.t) showed 0.11 folds (11%) in comparison with control.

A sudden elevation of 0.59 folds (59%) (415.27 µM hydroperoxy dienes / g. f.w.t) was observed in mechanical damage studies in day 0 when compared to control (260.72 µM hydroperoxy dienes / g.f.w.t). Activity decreased showing 0.37 folds (37%) hike (412.36 µM hydroperoxy dienes / g.f.w.t) in test samples against control (300.72µM hydroperoxy dienes / g.f.w.t) on day 1. The variations decreased further days reaching test sample (406.18 µM hydroperoxy dienes / g. f.w.t) showing 0.18 folds (18%) hike over control (344.36 µM hydroperoxy dienes / g.f.w.t) on day 3. On day 5 there is a negligible variation seen between test (489.81 µM hydroperoxy dienes / g.f.w.t) and control (478.9 µM hydroperoxy dienes / g.f.w.t) where test samples activity is more by 0.02 folds (2%). An almost similar findings were observed on day 7 as test (402.9 µM hydroperoxy dienes / g .f.w.t) shows 0.08 folds (8%) hike over control (369.81 µM hydroperoxy dienes / g.f.w.t). On day 9, test sample (345.09 µM hydroperoxy dienes / g.f.w.t) showed 0.12 folds (12 %) hike over control (308.18 µM hydroperoxy dienes / g.f.w.t). on day 10, test sample (334.54 µM hydroperoxy dienes / g.f.w.t) was elevated by 0.15 folds (15 %) over control (289.81µM hydroperoxy dienes / g.f.w.t) (Fig 1)

The obtained results clearly indicates that during drought there is a slow response induced in LOX activity during the initial days and later on reaches a maximum on day 7 and reduced to lower values on further sampling days. Whereas a sudden increase was observed during initial days in LOX activity when plant was mechanically wounded and later on decreased on further sampling days.

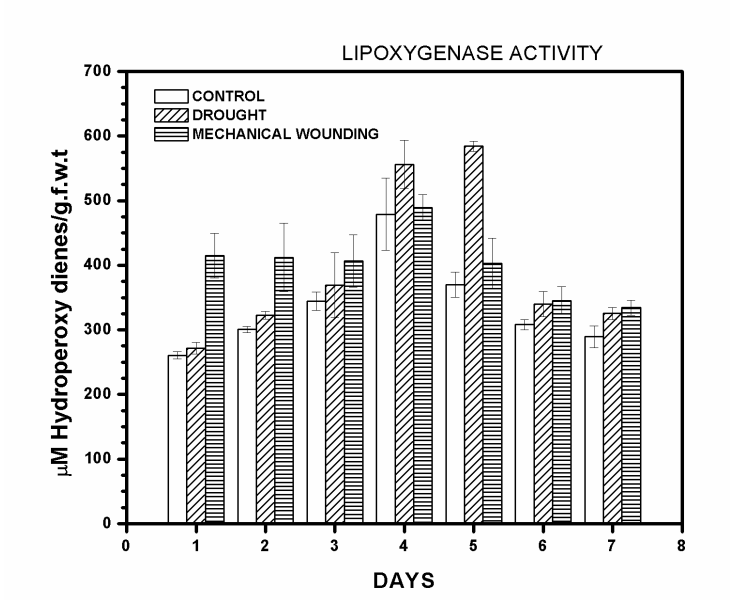

Figure 1: LOX activity in Horse gram plants during drought stress and mechanical wounding against unstressed control plant. LOX activity is expressed in micro molar hydroperoxy dienes per gram of fresh weight tissue. (µM Hydroperoxy dienes / g .f.w.t)

These variations observed in LOX activity was clearly explained by the fact that the lipids, the structural constituents of most cellular membranes serve as the substrate for LOX enzyme, undergoes peroxidation in various levels as per the need of the plant during growth and stress exposed conditions. In *Arabidopsis*, lipids are oxidized through the 13-LOX and 13-hydroperoxide lyase (13-HPL) pathway [22] and these 13-LOX and 13-HPL pathway was significantly down-regulated [23] for the survival of the plant was reported. The same is reported under the dehydration condition in chrysanthemum, which could maintain the membrane integrity to reduce damages caused by drought. Regulation of the LOX pathways during stress by the plants for its survival emphasizes the role of LOX during stress management. Drought and mechanical wounding stress leads to oxidative damage through an increase in amounts of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals. The ROS interact with a wide range of molecules, causing pigment co oxidation, lipid peroxidation, membrane destruction, protein denaturation, and DNA mutation. ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in the plasma lemma or intracellular organelles, which finally leads to the leakage of cell [24].

H_2O_2 quantification

During drought stress an insignificant changes in H_2O_2quantity was observed on day 0. Test sample (21mMH_2O_2 g^{-1}f.w.t) showed 0.02 folds (2%) hike over control (20.5mMH_2O_2 g^{-1}f.w.t). On day 1 there is a remarkable increment of 0.86 folds (86%) of test sample (42 mM H_2O_2 g^{-1}f.w.t) over control (22.5Mm H_2O_2 g^{-1}f.w.t). An increment by 0.74 folds (74%) was observed in test sample (44.5mMH_2O_2 g^{-1}f.w.t) over control (25.5mMH_2O_2 g^{-1}f.w.t) on day 3. H_2O_2 accumulation rate was slightly higher on day 5 as the test sample (52.5mMH_2O_2 g^{-1}f.w.t) showed 0.94 folds (94%) hike over the control (27.mMH_2O_2 g^{-1}f.w.t). Test samples (71.3mMH_2O_2 g^{-1}f.w.t) on day 7, (plant starts to wilt) showed a hike by 1.69 folds over control (26.5mMH_2O_2 g^{-1}f.w.t). Further sampling on day 9 showed a reduction in accumulation of H_2O_2 in test sample (66.9mMH_2O_2 g^{-1}f.w.t) by 1.57 folds over control (26.mMH_2O_2 g^{-1}f.w.t). The values corresponding to increase in H_2O_2 quantity continues to reduce on day 10 showing 1.49 folds increment in test sample (62.4mMH_2O_2 g^{-1}f.w.t) over control(25mMH_2O_2 g^{-1}f.w.t).

In Mechanical wounding studies, H_2O_2 quantities showed reduced elevations compared to drought. On day 0 test sample (27.5mMH_2O_2 g^{-1}f.w.t) showed 0.34 (34%) increment over control (20.5mMH_2O_2 g^{-1}f.w.t). Accumulation of H_2O_2 decreased on day 1 test sample (28.5mMH_2O_2 g^{-1}f.w.t) showed 0.26 folds (26 %) increase over control (22.5mMH_2O_2 g^{-1}f.w.t) and it continues to decline showing only 0.09 folds (9%) increment in test sample (28mMH_2O_2 g^{-1}f.w.t) over control (25.5mMH_2O_2 g^{-1}f.w.t) on day 3. Changes between test sample (27.5mMH_2O_2 g^{-1}f.w.t) and control (27mMH_2O_2 g^{-1}f.w.t) become negligible as there is only 1% variation between them on day 5. More or less similar findings were observed on day 7, 9 and 10 as the test samples showed very little variations compared to control (Fig 2).

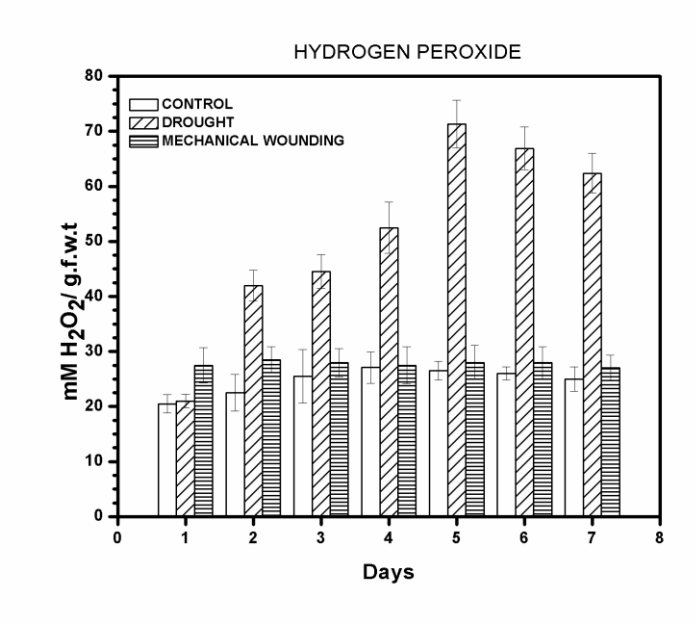

Figure 2: Hydrogen peroxide quantification in Horse gram plants during drought stress and mechanical wounding against unstressed control plant. Hydrogen peroxide quantity is expressed in mM Hydrogen peroxide per gram of fresh weight tissue (mM H_2O_2 /g f.w.t)

The H_2O_2 levels remained slightly higher in test sample in comparison with control and increased rapidly to significant levels on day 1 and it continued to increase in the further samples. During mechanical wounding, there was an initial hike in the H_2O_2 levels and later on decreases reaches minimal values on day 10. H_2O_2 is the most stable, active, toxic and destructive causing deleterious effect in plantsgenerated by the ROS. During drought stress, water loss was avoided by the plant by stomatal closure. Availability of CO_2 for photosynthesis is greatly reduced due to this stomatal closure, a major temporary adaptive response in plants. Reduced CO_2levels in leaves forces electrons in photo systems to be get misdirected and produce reactive oxygen species such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) in photosynthetic cells. Activated oxygen species production and the antioxidant quenching activity is balanced in a proper manner which helps in the survival of the plant, upset of this balance often leads to oxidative damage. Metabolic processes in plants produce mainly four types ROS among which H_2O_2 is a stable one. Reports regarding irreversible inactivation of soybean LOX-1, which prefers anionic substratesby hydrogen peroxide (H_2O_2) establishes a relation between LOX enzyme and H_2O_2 [25].

Lipid peroxidation

During drought stress an insignificant changes in Malondialdehyde content was observed on day 0. Test sample (3.9 mM MDA/ g f.w.t) showed 0.11 folds (11%) hike over control (3.5mM MDA/ g f.w.t). On day 1 there is an increment of 0.11 folds (11%) in test sample (4.7mM MDA/ g f.w.t) over control (4.2mM MDA/ g f.w.t). Further increment was observed up by 0.4 folds (40%) in test sample (6.3mM MDA/ g f.w.t) over control (4.5mM MDA/ g f.w.t) on day 3. Malondialdehyde content remained slightly higher on day 5 as the test sample (6.93mM MDA/ g f.w.t) showed 0.5 folds (50%) hike over the control (4.6mM MDA/ g f.w.t). Test samples (9.13mM MDA/ g f.w.t) on day 7, (plant starts to wilt) showed a hike by 0.86 folds (86%) over control (4.9mM MDA/ g f.w.t). Further sampling on day 9 showed a reduction in accumulation of Malondialdehyde content in test sample (8.45mM MDA/ g f.w.t)by 0.65 folds (65%) over control (5.1mM MDA/ g f.w.t). The values corresponding to % of increase in Malondialdehyde content continues to reduce on day 10 showing 0.55 folds (55%) increment in test sample (7.95mM MDA/ g f.w.t)over control (5.1mM MDA/ g f.w.t).

During mechanical wounding a drastic change in Malondialdehyde content was observed on day 0. Test sample (7.89mM MDA/ g f.w.t) showed 1.25 folds hike over control (3.5mM MDA/ g f.w.t). On day 1 there is an increment of 0.5 folds (50%) in test sample (6.34mM MDA/ g f.w.t) over control (4.2mM MDA/ g f.w.t). Further increment was observed up to 0.33 folds (33%) in test sample (6.011mM MDA/ g f.w.t) over control (4.5mM MDA/ g f.w.t) on day 3.

Malondialdehyde content remained slightly higher on day 5 as the test sample (5.97mM MDA/ g f.w.t) showed 0.29 folds (29%) hike over the control (4.6mM MDA/ g f.w.t). Test samples (5.42mM MDA/ g f.w.t) on day 7, (plant starts to wilt) showed a hike by 0.1 folds (10%) over control (4.9mM MDA/ g f.w.t). Further sampling on day 9 showed a reduction in accumulation of Malondialdehyde content in test sample (5.38mM MDA/ g f.w.t)by 0.05 folds (5%) over control (5.1mM MDA/ g f.w.t). The values corresponding to increment in Malondialdehyde content continues to reduce on day 10 showing 0.04 folds (4%) increment in test sample (5.35mM MDA/ g f.w.t)over control (5.1mM MDA/ g f.w.t) (Fig3).

The MDA content is a product of lipid peroxidation and considered as an indicator of oxidative damage. Lipid peroxidation occurs due to direct attack of ROS on lipids and proteins and their destruction.The generation of lipid peroxides leads to the alterations in polyunsaturated fatty acids of the cell membranes shows profound effect on the structural and functional properties leading to an increase in membrane permeability and membrane bound proteins inactivation [26].

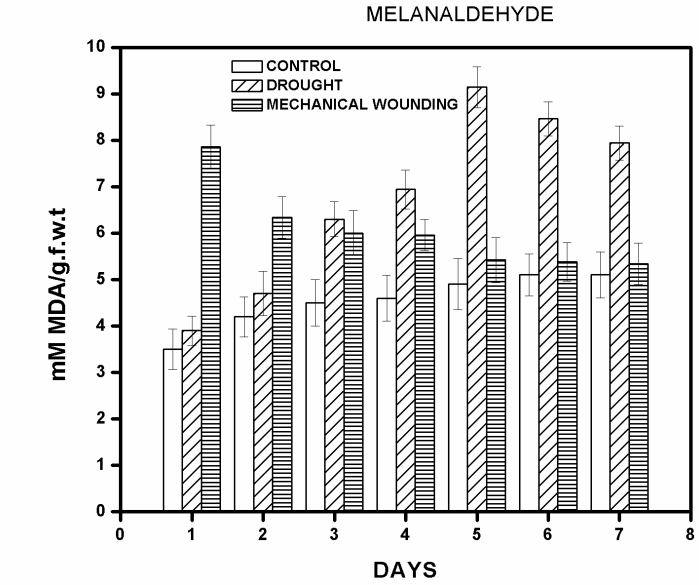

Figure 3: Malondialdehyde content generated due to lipid peroxidation in Horse gram plants during drought stress and mechanical wounding against unstressed control plant. Malondialdehyde content is expressed in milli molar of Malondialdehyde generated per gram of fresh weight tissue (mM MDA/ g f.w.t)

In the present study, an increase in MDA content was insignificant during the initial stages of drought stress and started to increase rapidly as the stress condition of the plant continues and reaches maximum at the wilting day (day 7). In mechanical wounding, rapid accumulation of MDA was observed initially and decreased later on as the stress continues. These results suggest that the scavengers of ROS, the antioxidativeenzymes respond variably to these different kinds of stress conditions.

Proline assay

During drought stress an insignificant changes in proline content was observed on day 0. Test sample (4.2µg proline / g f.w.t) showed 0.05 folds (5%) hike over control (4.0 .µg proline / g f.w.t). On day 1 an increment by 0.09 folds (9%) in test sample (4.7 .µg proline / g f.w.t) over control (4.3µg proline / g f.w.t) was observed. Further increment was observed up to 0.11 folds (11%) in test sample (5.8µg proline / g f.w.t) over control (5.2µg proline / g f.w.t) on day 3. Proline content remained slightly higher on day 5 as the test sample (7.6µg proline / g f.w.t) showed 0.35 folds (35%) hike over the control (5.6µg proline / g f.w.t). Test samples (8µg proline / g f.w.t) on day 7, (plant starts to wilt) showed a hike of 0.35 folds (35%) over control (5.9µg proline / g f.w.t). Further sampling on day 9 showed a slightly higher in accumulation of proline content in test sample (8.2µg proline / g f.w.t) by 0.36 folds (36 %) over control (6.0 µg proline / g f.w.t). The values corresponding to increment in proline content tend to reduce on day 10 showing 0.31 folds (31%) increment in test sample (8µg proline / g f.w.t) over control (6.1µg proline / g f.w.t). During mechanical wounding drastic and significant changes in proline content was observed on day 0. Test sample (8.6µg proline / g f.w.t) showed 1.15 folds hike over control (4.0µg proline / g f.w.t).

On day 1, an increment of 1.04 folds in test sample (8.8µg proline / g f.w.t) over control (4.3µg proline / g f.w.t) was observed. Proline content decreased slightly accounting to 0.51 folds (51%) in test sample (7.9µg proline / g f.w.t) over control (5.2µg proline / g f.w.t) on day 3. Proline content reduced slightly on day 5 as the test sample (7.7µg proline / g f.w.t) showed 0.37 folds (37%) hike over the control (5.6 µg proline / g f.w.t). Test samples (7.3 µg proline / g f.w.t) on day 7, (plant starts to wilt) showed a hike by 0.23 folds (23%) over control (5.9µg proline / g f.w.t). Further sampling on day 9 showed a reduction in accumulation of proline content in test sample (6.9µg proline / g f.w.t) by 0.15 folds (15 %) over control (6.0µg proline / g f.w.t). The values corresponding to increment in proline content continues to reduce on day 10 showing 0.03 folds increment in test sample (6.3µg proline / g f.w.t)over control (6.1µg proline / g f.w.t) (Fig4).

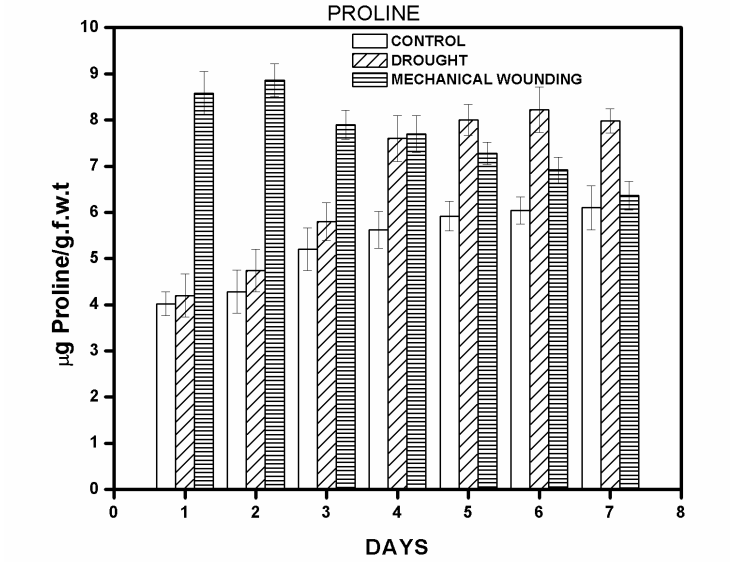

Figure 4: Proline content in Horse gram plants during drought stress and mechanical wounding against unstressed control plant. Proline content is expressed in micrograms of proline produced per gram of fresh weight tissue (µg proline / g f.w.t)

Water deficiency and increased cellular proline levels were strongly correlated in the survival of the organisms ranging from bacteria to higher plants during stress prone conditions. It may also serve as an organic nitrogen reserve that can be utilized during recovery. In *Lathyrus sativus*, a hardy grain legume which can withstand drought, high proline accumulation was observed in leaves and roots under water stress. Although proline can be synthesized from either glutamate or ornithine; glutamate is the primary precursor in osmotically stressed cells. The biosynthetic pathway consists of two important enzymes, viz. pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase. Transcripts corresponding to both cDNAs accumulate in response to NaCl treatment. Both these regulatory steps are keys to developing strategies for overproducing proline in a selected plant species. Accumulation of osmolytes during drought stress by plants regular phenomenon observed in plants. Prolines, the osmolytic biomolecules not only stabilizes the proteins and maintain cell membrane structure but also acts as free radical scavengers and stress related signal molecules [27]. Proline's role in cellular homeostasis during redox balance and in energy status in *Arabidopsis* was reported [28]. Recent findings regarding its tissue specific synthesis, maintaining higher NADP/NADPH ratio at relatively low water potentials shows its specific activity in promoting the growth of the plant tissue. In our present study, there is a high accumulation of proline content during the initial days during mechanical damage and a significantly lower level of accumulation was observed during drought stress.

CONCLUSION

Inour present study we conclude that plant responds to different stress conditions as per the need of the plant. A rapid accumulation of biochemical entities of our study was observed during mechanical wounding in comparison with the drought stress. This study also concludes the role of Lipoxygenase enzyme activity in producing the ROS in differing manner in response to the exposure of the plant to different kinds of stress.

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