

Title

The Effect of Drought Stress on The Activity of Monoamine Oxidase in Different Wheat Cultivars

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Abstract

Drought is a major stress factor for plants leading to detrimental effects on the growth and yield of plants. As a consequence of drought stress, plants normal equilibrium get disturbed thereby leading to the host of changes at physiological and biochemical level. Monoamine oxidases (MAOs) are known to limit ROS under different stress perturbations, but research showing how boiling stable MAOs is regulated under drought is still a matter of conjuncture. Through this work, the changes in lipid peroxidation and boiling soluble MAOs were studied in the four wheat cultivars. Concomitant analysis of MDA contents and boiling soluble MAOs enzymes provided a view of the detoxifying potential under conditions of stress. Markers of oxidative stress viz. MDA content decreased under drought treatment depending upon the genotype. Based upon the results, it can be inferred that HD 2932 has more ability to combat the oxidative stress by increasing the biological anti-oxidative boiling stable monoamine oxidase activity.

Key Words: Antioxidants, Boiling soluble proteins(BSPs), Monoamine oxidase

Introduction

The big paradoxical question of: How do we adapt to and plan for uncertainty in the environment is becoming a nightmare for the farmers as well as agricultural biotechnologists throughout the world. Growth and yield of different crops is dramatically affected by plethora of environmental constraints due to regular changes in the weather pattern, with drought being the most devastating environmental cue decreasing crop yield greater than other abiotic stress factors [1]. It is anticipated that due to global warming, water deficit conditions would intensify, with rise from one to thirty percent in an intense drought prone land till the end of 21st century [2].

ROS are unavoidable products of the metabolic pathways and electron transport systems linked to the cell membrane [3]. However, under the effect of stress conditions, the altered redox equilibrium results in the aggravated production of ROS (O_2^- , H_2O_2 , OH^\cdot) [4]. The elevated levels of ROS oxidize proteins, damage nucleic acids, peroxides lipids, activate the apoptotic pathway ultimately leading to the cell death [5]. MDA (Malondialdehyde), being the most prominent lipid breakdown product, is regarded as a significant marker for oxidation of the lipids present in cell membranes [6]. To tackle the detrimental effects of ROS, plants have antioxidative machinery consisting of various enzymatic and non-enzymatic antioxidant molecules. Amine oxidases (AOs) including Monoamine oxidase (FAD dependent) are also considered to be a part of anti-oxidative machinery implicated in defense response besides stiffening of the cell wall [7]. Monoamine oxidase catalyzes the amines oxidation using O_2 as an electron acceptor resulting in the formation of the corresponding aldehyde and ammonia.

Few stress-induced antioxidant proteins are highly hydrophilic and have the tendency to remain stable and soluble even after the treatment of boiling, henceforth these are termed as “boiling soluble proteins” (BSPs), a term coined initially by [8]. Previous research findings have suggested that these BSPs epitomize less than one percent of the total protein content. Nevertheless, it signifies one of the prominent proteome part involved in imparting tolerance against abiotic and biotic constraints [9]. There have been several reports that authenticate the role of amine oxidases under stress but the role played by MAOs under oxidative stress conditions is still in its infancy. [10,11] documented the potential role of various amine oxidases in plants during biotic or abiotic conditions. Keeping this in mind, the study was

undertaken so as to have an insight into the role of boiling soluble MAO under different stress conditions followed by recovery in wheat cultivars. In present study, endosperms and embryos were extracted from four wheat cultivars at various drought intensities (2 h, 6 h, 24 h, and 48 h) followed by relief of stress after post stress stage.

Materials and methods

Seedlings growth

Seeds of four wheat (*Triticum aestivum* L.) cultivars (HD2851, HD2932, HD2967 and HD3086) were selected for experimental purposes. The surface sterilization of the seeds was done using chemical disinfectants (mercuric chloride and 70% ethanol [12]. After this, the seeds were imbibed for 6 hr and then placed in Petri plates for germination at 25 ± 1°C for 24 hr. Stress was imposed by withholding water supply for 2hr(D2), 6hr(D6), 24hr(D24), 48hr(D48). After 48hr of drought stress treatment, stress was relieved by rewatering the seeds for 4hr (4PS). After different treatments, tissues were separated into embryo and endosperm and then kept for further experimental analysis. The Water content (WC) was calculated from the equation: **WC = fresh weight-dry weight/dry weight**

Boiling stable proteins extraction

Plant tissues were ground to fine slurry using 50 mM Tris- HCl buffer (pH-7.0). After centrifugation, some part of supernatant was collected and was labelled as untreated samples. The remaining supernatant was boiled for 10 minutes in boiling water bath, immediately kept in ice for 5 minutes and again centrifuged, then supernatant was collected which was labelled as the treated samples and stored at -20°C for further analysis. The protein concentration was measured spectrophotometrically according to the method of [13] utilizing BSA as a standard.

Lipid peroxidation

Trichloroacetic acid (TCA)	10%
Thiobarbituric acid (TBA)	0.25%

Lipid peroxidation was estimated by the level of malondialdehyde (MDA) produced by the thiobarbituric acid reaction [14].

Monoamine oxidase activity:

The level of MAO activity in plant tissues was determined spectrophotometrically by the conversion of benzylamine to benzaldehyde following the method of [15] with some modifications. 1 ml reaction mixture contains (800- X) μ l H₂O, 5 mM benzylamine (100 μ l), 100 mM phosphate buffer (100 μ l) pH 7.2 and 120 μ g protein sample (X). The formation of 0.15 μ mol benzaldehyde results in an increase in absorbance at 250 nm of 1 unit. Therefore, specific activity was expressed as the mmol of benzaldehyde formed $\text{min}^{-1} \text{mg protein}^{-1}$ ($\epsilon = 13800 \text{ M}^{-1} \text{ cm}^{-1}$).

RESULTS

Variation in water content (Wc):

Water Content was determined in four cultivars of wheat (HD 2932, HD 2967, HD 2851, HD 3086). In embryo (Figure: 2-A) as compared with control, In cultivar HD 2851, a tremendous reduction in water content was observed at all drought stress stages of 2 hr, 6 hr, 24 hr, 48 hr. However, In cv HD 2932 and HD 3086 a significant decrease in drought stress stages was observed at 24 h and 48 hr of drought stress stages. Contrary to this, in cv HD 2967 a significant decrease in water content was observed at 6 hr and 48 hr of drought stress stages. upon relieving stress after 48 hr of drought stress marked increase in water content was observed in all cultivars.

In Endosperm (Figure: 2-B) Water Content value variable in a cultivar and drought stress dependent manner. As compared with control, In cv 2851 and HD 3086 significant decrease in water content was observed at 24 hr and 48 hr of drought stress stage contrary to this , in cultivars HD 2932 and HD 2967 significant decline in water content was observed at all drought stress stages. Upon relieving stress after 48 hr of drought stress, a significant increase in water content was observed in all cultivars.

Variation in protein content : TPC was determined in four cultivars of wheat (HD 2932, HD 2967, HD 2851, HD 3086). In embryo (Figure 3-A), as compared with control, in cv HD 2851 substantial increase in TPC was observed at 2 h, 6 h, 24 h, of drought stress. However as the severity of stress increased to 48 h, a marked significant decline in TPC was detected but it was still higher than control. In cultivar HD 2932 a significant increase was observed at

one day and two days of drought stress. On the other hand, in cultivar HD 3086 a significant increase was observed in TPC value at all stages of drought stress except at 2 h of drought stress. Contrary to this in cv HD 2967 a significant decrease in TPC value was observed at 6 h, 24 h, of drought stress. Upon relieving stress after two days of stress treatment, a significant decline in TPC value was observed in the cvs HD 2851, HD 2967, HD 3086. Conversely, in the cv HD 2932 substantial enhancement was observed in TPC at 4 h of post stress.

In endosperm (Figure 3-B) TPC values were variable in a cultivars and drought stress stage dependent manner. In cv HD 2851 TPC decreased at all the stages with maximum decrease at 48h. In cv HD 2932, in comparison to the control, a significant decrease in TPC was observed only at 6 h of drought stress. Contrary to this, in cv HD 2967 marked significant increase in TPC was observed at 6 h of drought stress. However, in cv HD 3086 significant increase in TPC was observed at 2h and 6h stage of stress imposition. However, as the stress intensity increased at two days of drought stress, tremendous decrease in TPC was noticed. Upon relieving stress after two days of drought stress treatment, a significant decrease in TPC was observed in cultivar HD 3086. While in the remaining cultivars, it remained unchanged.

Change in MDA : MDA was determined in four cultivars of wheat (HD 2932, HD 2967, HD 2851, HD 3086). In Embryo (Figure 4-A) MDA values were variable in cultivar and drought stress stage dependent manner. In cv HD 2851, as compared to control, a marked decline in MDA was observed at 2 h of drought stress. However, as the severity of drought stress increased a marked significant increase in MDA content was observed at one day, two days of drought stress. In cv HD 2932 MDA content increased significantly at two days of drought stress stage. On the other hand in cv HD 2967, significant decline in MDA content was observed at low (6 h) and (medium) 24 h of drought stress stages. However in cv HD 3086 MDA content declined significantly at 24 h of drought stress stage. Upon relieving stress after 48 h of drought stress a significant decrease in MDA content was found in all cultivars.

In endosperm, MDA (Figure- 4 B) values were observed in four different cultivars of wheat. In cv HD 2851 and cv HD 2932, as compared to control, an increase in MDA content was found at 48 h of drought stress stage. However in cv HD 3086, decrease was observed in MDA value at all stages of drought stress, except at 6 h of drought stress stage. Contrary to this in cv HD 2967, as compared to control, an increase in MDA value was observed at 48 h of the drought stress stage. Upon relieving stress after 48 h of stress stages, in cultivars HD 2851, HD 2932 and HD 3086 MDA content increased significantly.

Change in MAO activity :

BsMAO activity was determined in four cultivars of wheat HD 2932, HD 2967, HD 2851 and HD 3086. In embryo (Figure 5-A), as compared with control, in cultivars HD 2851, decrease in BsMAO activity was recorded at 6 h, 24 h, 48 h drought stress stages, while in cv HD 3086, a tremendous decline in BsMAO activity was observed at 6 h of drought stress stage. Contrary to this, in cv. HD 2932 a significant decrease in BsMAO activity was observed at 6 h of drought stress stage, Contrary to this in cv HD 2967 significant increase in BsMAO activity was observed at 24h and 48 h of drought stress stages, But BsMAO activity decreased significantly at 6 h and 24 h of drought stress stages in this cultivar. Upon relieving stress after 48 h of drought stress, in cv HD 2851 a significant decrease in BsMAO was observed. Contrary to this, in cv HD 2967 as compared with control, BsMAO activity increased significantly.

In endosperm (Figure 5-B), as compared with control, in cv HD 2851 BsMAO value increased significantly at one day and two days of drought stress stages, while in cv HD 2932 BsMAO value, increased tremendously at 2 h and 6 h of drought stress stages. contrary to this, in cv HD 3086 a significant decrease in BsMAO was observed at 2 h of drought stress stage. On the other hand, in cv HD 2967, nil value of BsMAO was observed at all drought stress stages. Upon relieving stress after 48 h of drought stress, in cultivar HD 2851, marked decline in BsMAO activity was observed. Contrary to this, in cv HD 3086, we observed a significant increase in BsMAO activity.

Discussion

Variation in water content: Water Content was determined in four cultivars of wheat HD 2932, HD 2967, HD 2851, HD 3086. In the present study, in embryo and endosperm (figure 2- A and B) as compared with control, water content decreased significantly under drought in a stress stage dependent manner in all cultivars. Previous research findings also indicated that in response to drought, WC reduced in different crop species ([16,17]. Upon relieving stress after 48 h, a significant increase in water content was observed.

Variation in TPC:

A study by [18] has reported that stress conditions affect expression of genes and synthesis of proteins. In the present study it is observed that change in TPC were variable in cultivar, tissue dependant manner. TPC was observed in both embryo and endosperm of four different cultivars under drought stress. In embryo (Figure 3-A) of wheat cultivars as compared with

control, a significant increase in TPC was observed in cultivars HD 2932, HD 2851, HD 3086 at the drought stress stages. Consistent with these finding, [19] also reported that the leaf proteins increased during severe drought. Contrary to this in cv.HD 2967 TPC content decreased significantly at 6h, 24 h of drought stress stage. The heightened proteolytic and catabolic activity along with lethal ROS levels result in the degradation of protein. The variations in total soluble proteins under drought stress were consistent with the findings of [20] in maize. During post stress period as compared with control TPC content decreased considerably in all cultivars except 2932 . The reduction in TPC has been previously reported in other species like mulberry[21].

In Endosperm, as compared with control a significant decrease in TPC content was found in HD 2851 and HD 2932 cultivars at all drought stress stages. In agreement with the results [22] also reported decline in total protein content under water deficits. Contrary to this, in cultivar HD 3086 increase in TPC was observed at drought stress stages of 2 h and 6 h. [23] study reported augmentation in soluble protein content following water stress. The elevated TPC under drought conditions can be the result of activation of some genes coding for BSPs under drought stress conditions. Upon relieving stress, as compared with control, in cv 3086 TPC content decreased significantly.

Changes in MDA content :

In the present study, MDA content in embryo and endosperm is detected (Figure 4- A and B). In embryo of four wheat cultivars (HD 2932, HD 2967, HD 2851, HD 3086), In cultivars 2851 and 2932, in comparison to control, a remarkable decline in MDA content was observed. These results were in agreement with previous studies by [24],[25],[26], who found that low MDA levels are a result of tolerance towards drought in artichoke plans, common beans, and wheat, respectively. In cultivars HD 2851 and HD 2932, as the severity of drought increased , MDA content increased with increase in intensity of drought. It has been reported that water stress increased the lipid peroxidase productions in leaves of stressed *Phaseolus vulgarism* plants [27]. During post stress stage, as compared with control decrease in MDA content was observed in all cultivars.

In endosperm (Figure 4- B) of wheat cultivars as compared with control, in cultivars HD 2851, HD 2932 and HD 2967 MDA content increased significantly drought stress stage of 48 h. [28] reported that in Marigold, lipid peroxidation in both the leaves and petals increased in drought stress response. At post stress stages MDA content increased significantly in

cultivars HD 2851, HD 2932, HD 3086 and these findings corroborate the previous findings by [29] who also reported an increase in MDA content upon stress relief.

Change in MAO activity: In spite of the fact that different stress conditions induce the expression of different antioxidative enzymes, nevertheless the functional delinearity of boiling soluble MAOs under water stress and recovery has not been much highlighted. So in order to gain further insight into the biochemical role of boiling stable MAO (BsMAOs), we studied the effect of drought on the expression of BsMAO in different cultivars of wheat (HD 2932, HD 2967, HD 2851, HD 3086). In present study, in embryo (Figure 5-A) in cultivar HD 2967, a significant increase in BsMAO activity was observed at 24 h of drought stress. It is noteworthy here that the MDA level decreased in the cultivar 2967 at 24 h of drought stress so there was a negative correlation between MDA content and BsMAO activity. Based upon our findings, it is hypothesized that BsMAO might be acting as antioxidants to decrease the MDA content resulting from increased ROS under stress. On removal of drought stress after 48 h, in cv. HD 3086 BsMAO activity increased significantly. Previous study have also depicted that the activity of different antioxidants might be greater for the recovery period as compared to stress as observed in pea [30]. Contrary to this in cv. HD 2851, BsMAO activity decreased significantly.

In endosperms (Figure 5-B), a significant increase in BsMAO activity was observed in cultivars HD 2851, HD 2932, HD 3086 in a stage dependent manner. Previous findings have indicated that plant amine oxidases, including monoamine oxidases are involved in various defense mechanisms by the synthesis of different secondary metabolic compounds [31]. A major function of amine oxidases in plants has been proposed to be H₂O₂ production, which has a role in wound healing through lignification of the cell wall matrix by a restructuring phenomenon during normal growth as well as under stress n removal of stress after 48 h of drought stress in cv. HD 2967, BsMAO activity increased significantly. This throws light on the basic mechanisms that preserves the protein integrity besides enabling repair upon relief of stress. This emphasizes that stress induces the expression of various antioxidative enzymes which 'strengthens' the crops for future stress conditions and this seems to be fundamental for the recovery phase.

Conclusions

The findings of the study depicted that water stress induces the oxidative stress in different wheat cultivars. The damage to the wheat cultivars was there as seen by decreased WC, variable MDA levels (i.e., lipid peroxidation) and heightened BsMAO enzyme activities. Higher BsMAO activity confirmed their role for survival under drought-induced oxidative

stress. Findings of the study established enhanced BsMAO activities during oxidative stress in a plant genotype dependent manner. Among the four cultivars, under drought stress, HD 2932 displayed a much more pronounced antioxidative mechanisms and therefore, they were sheltered from the harmful effects of water stress even at an increased stress severity. Therefore, these findings could be utilized as biochemical marker while breeding oxidative stress tolerant high yielding crops in arid regions.

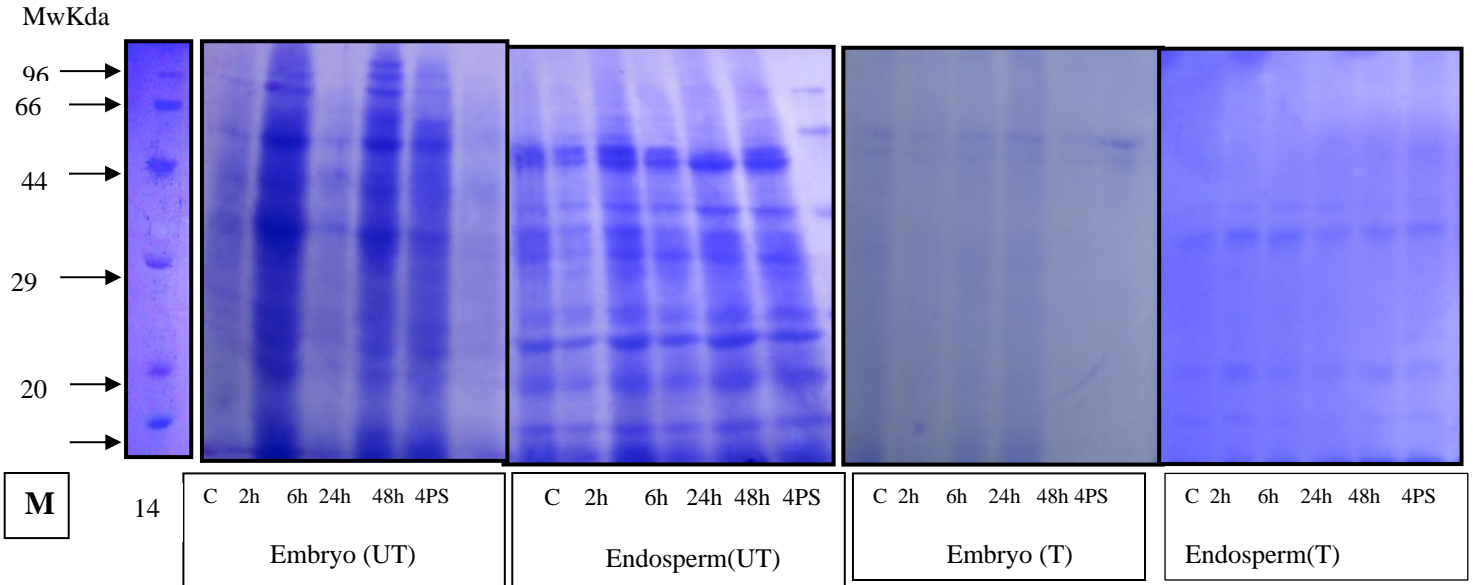
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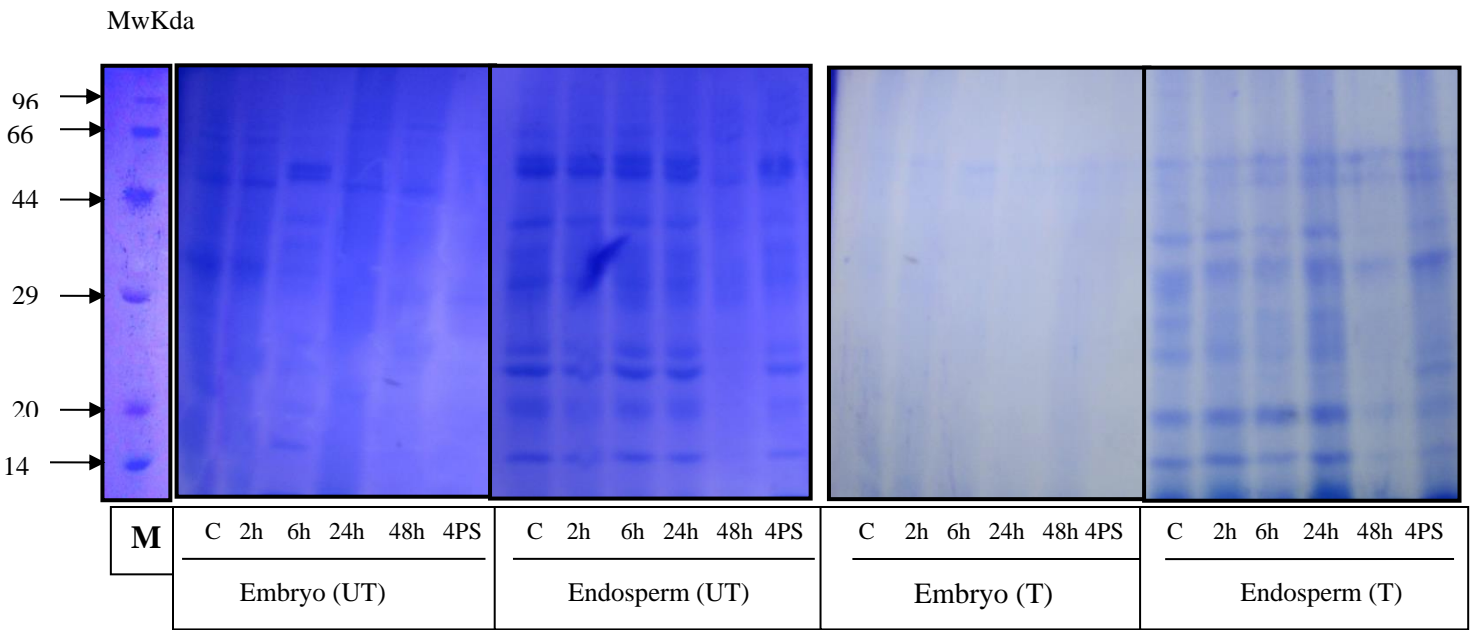
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HD2851



HD2932

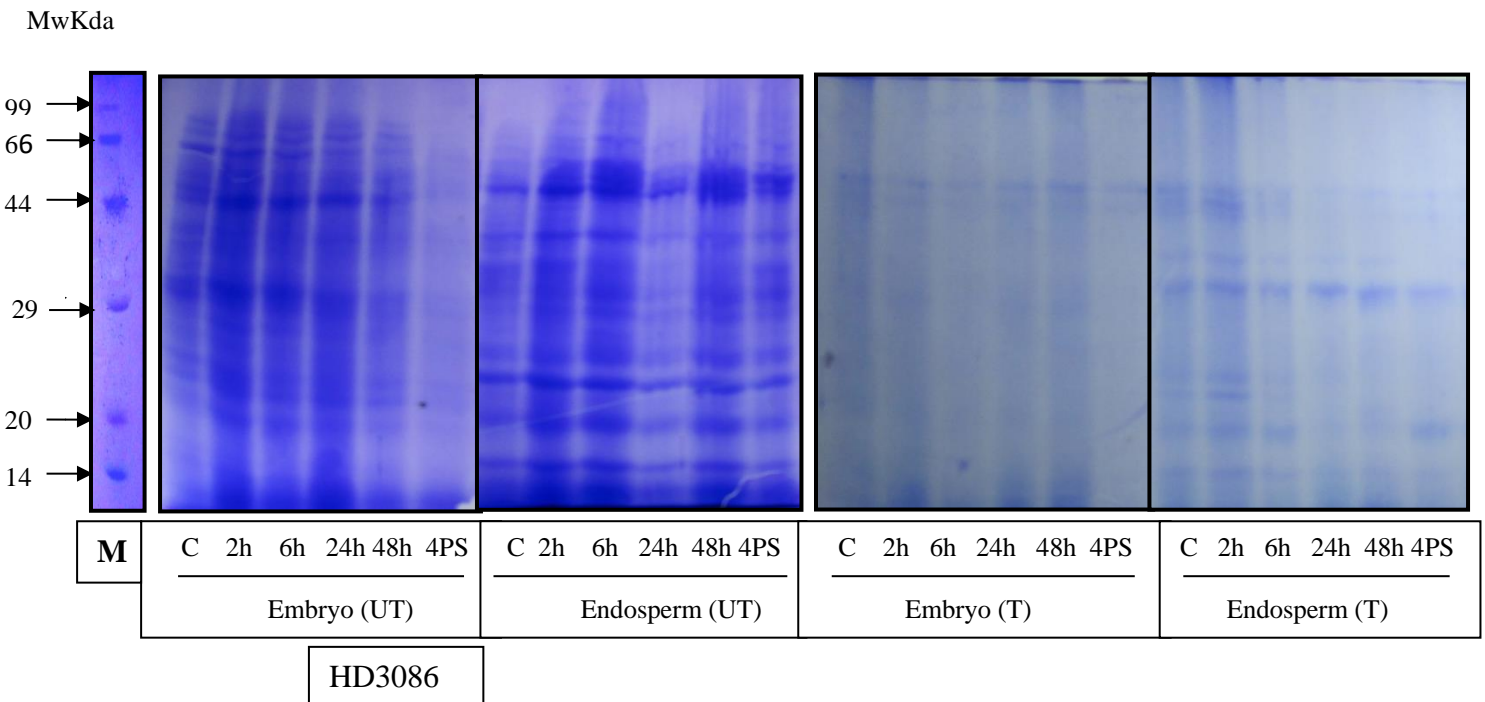
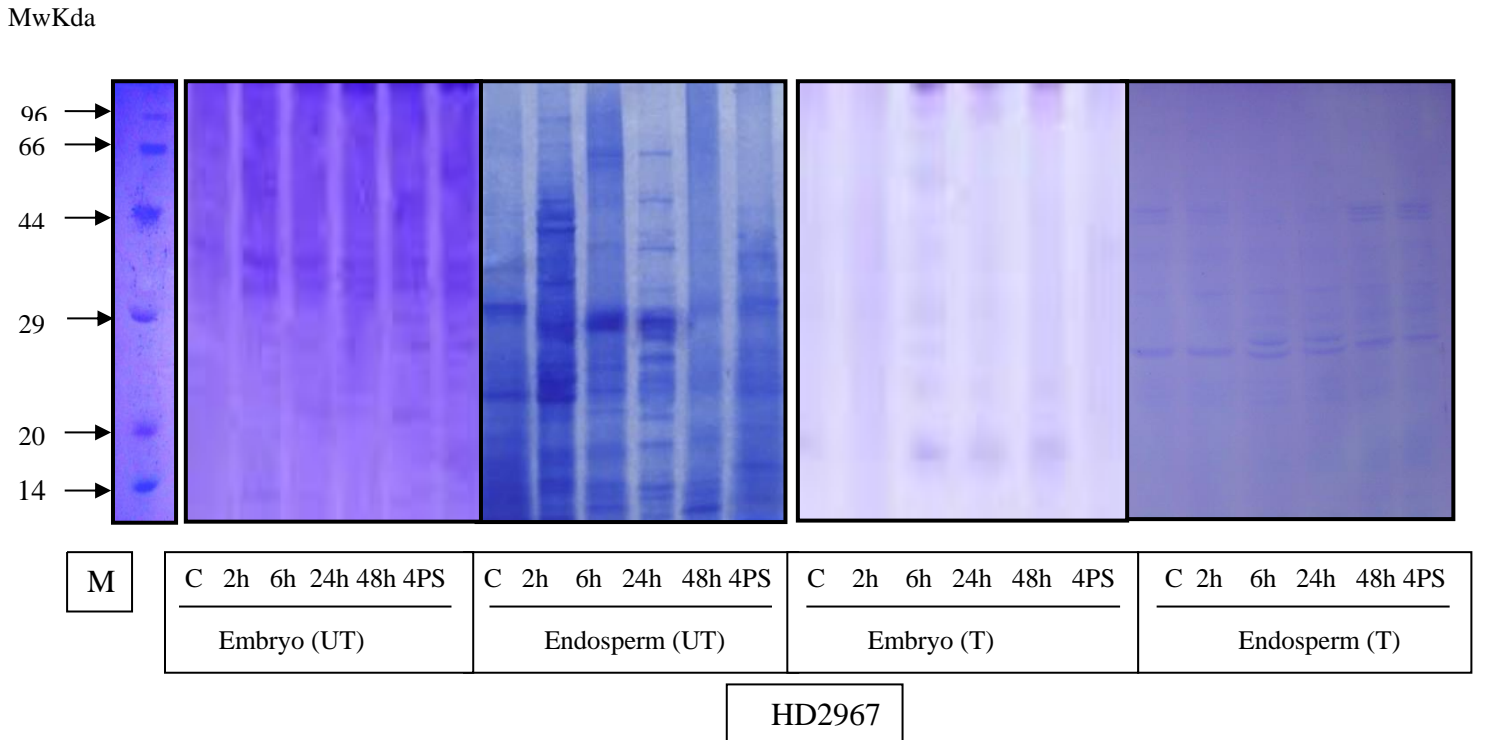
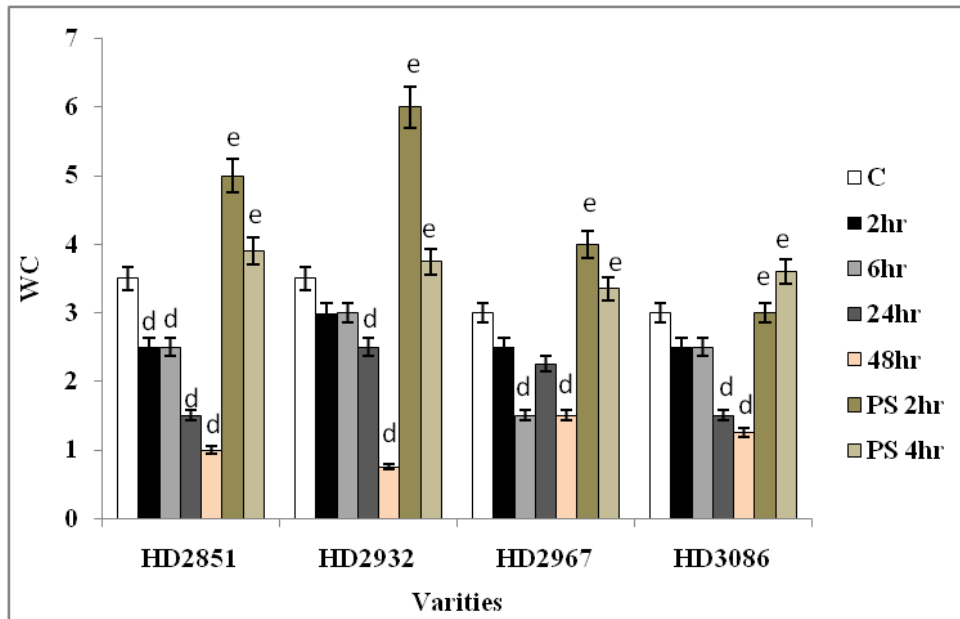
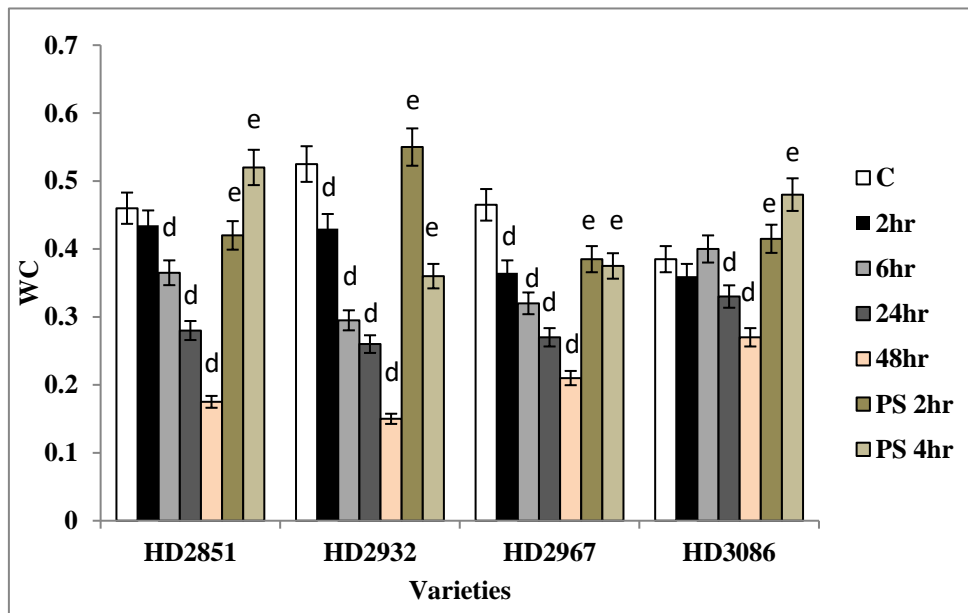


Fig. 1: An SDS PAGE profile of proteins in embryo and endosperm of four different cultivars (HD2851, HD2932, HD2967 and HD3086) of *T. aestivum* harvested at different stages (C, 2h, 6h, 24h, 48h) followed by recovery at 4PS respectively under drought stress conditions. Each lane loaded with 120µg of protein sample. Symbols used: M: marker, C: control, 2h: 2hours, 6h, 6hours, 24h: 24hours, 48h: 48hours of drought stress treatment, 4PS: 4 hours of post stress , UT: untreated protein samples, T: treated protein samples.

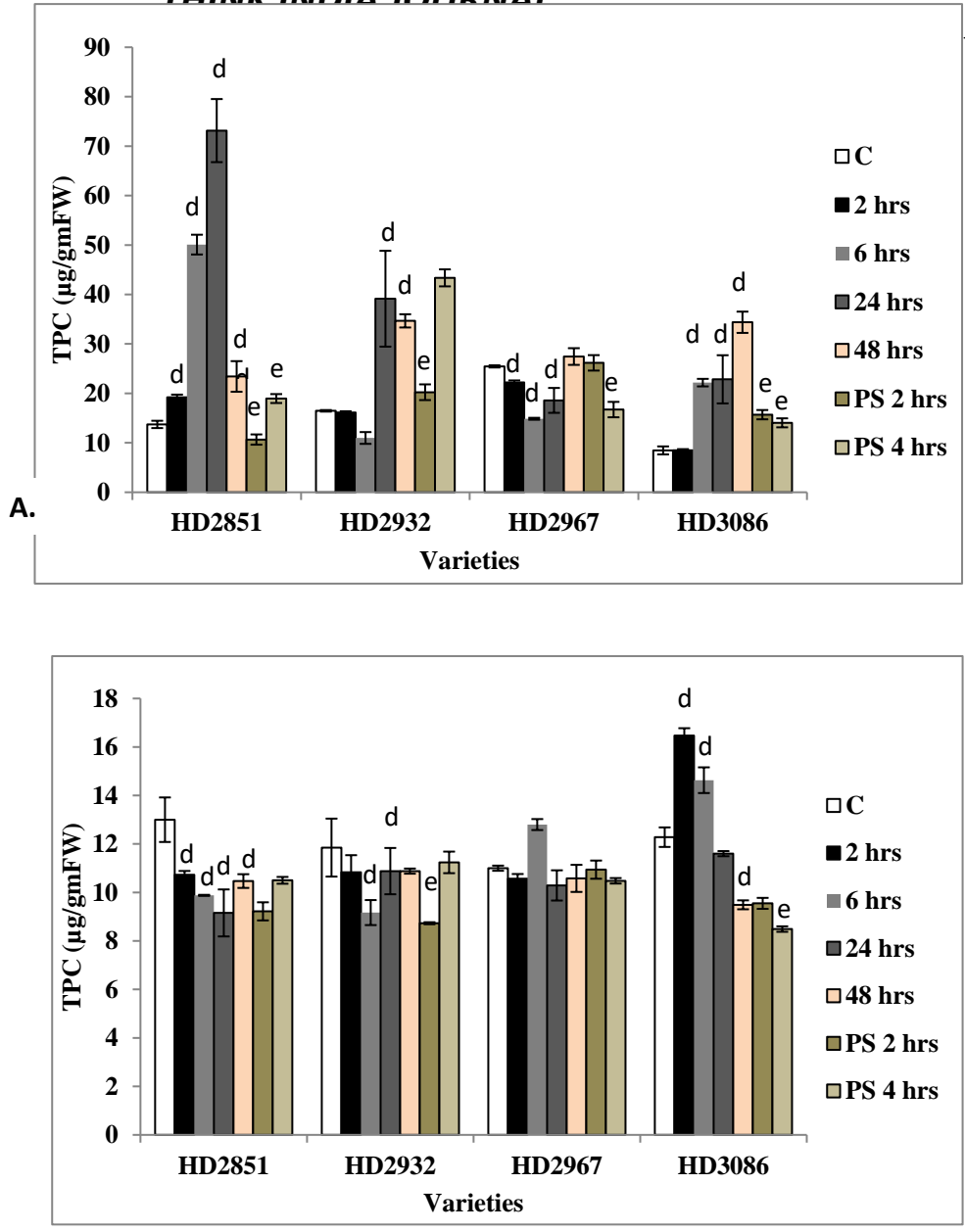


A.



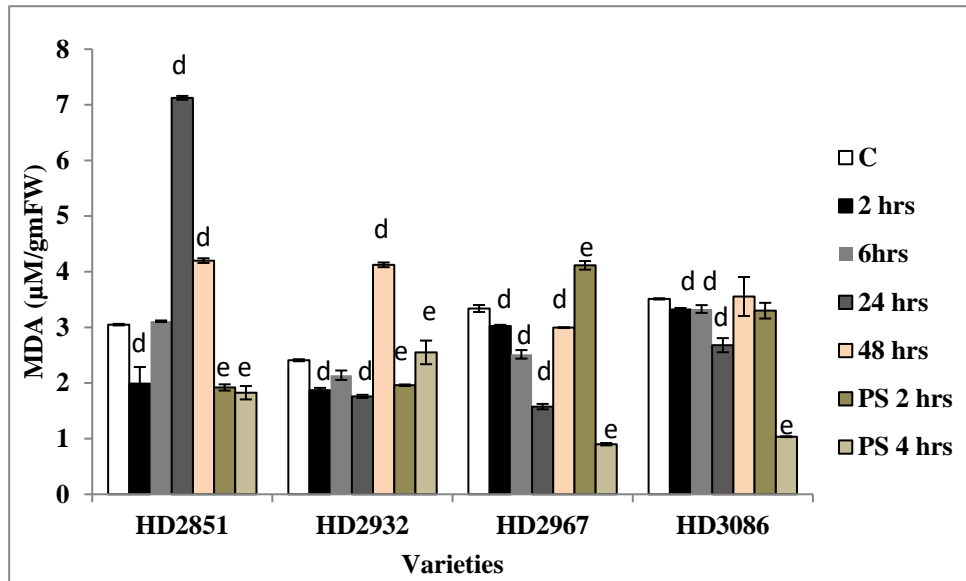
B.

Figure 2 : Water Content in Embryo(A) and Water Content in Endosperm(B) in four different wheat cultivars under control and different drought stress treatment. Symbol used: C-Control, 2hrs- 2hrs drought, 6hrs-6hrs drought, 24hrs-24hrs drought, 48hrs-48hrs drought, PS-Post Stress. Data shown are average ± SE. ^dindicates significant difference between C v/s 2hrs-48hrs at P≤0.05. ^eindicates significant difference between 48hrs v/s PS 2hrs-PS 4hrs at P≤ 0.05

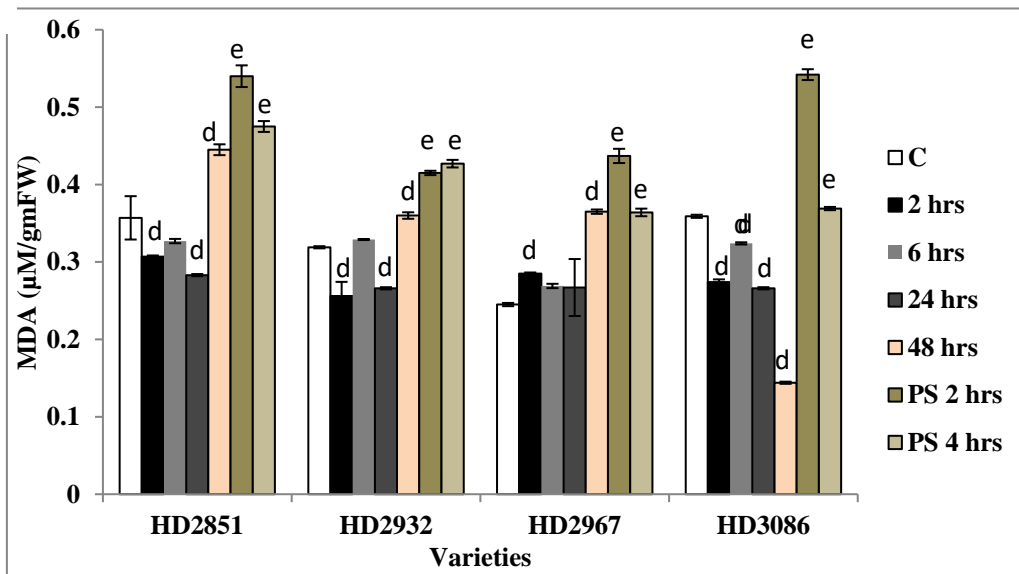


B.

Figure 3 : TPC in Embryo(A) and TPC in Endosperm(B) in four different wheat cultivars under control and different drought stress treatment. Symbol used: C-Control, 2hrs- 2hrs drought, 6hrs-6hrs drought, 24hrs-24hrs drought, 48hrs-48hrs drought, PS-Post Stress. Data shown are average \pm SE. ^dindicates significant difference between C v/s 2hrs-48hrs at $P \leq 0.05$. ^eindicates significant difference between 48hrs v/s PS 2hrs-PS 4hrs at $P \leq 0.05$.



A.



B.

Figure 4 : MDA Content in Embryo(A) and MDA Content in Endosperm(B) in four different wheat cultivars under control and different drought stress treatment. Symbol used: C-Control, 2hrs- 2hrs drought, 6hrs-6hrs drought, 24hrs-24hrs drought, 48hrs-48hrs drought, PS-Post Stress. Data shown are average \pm SE. ^dindicates significant difference between C v/s 2hrs-48hrs at $P \leq 0.05$. ^eindicates significant difference between 48hrs v/s PS 2hrs-PS 4hrs at $P \leq 0.05$.

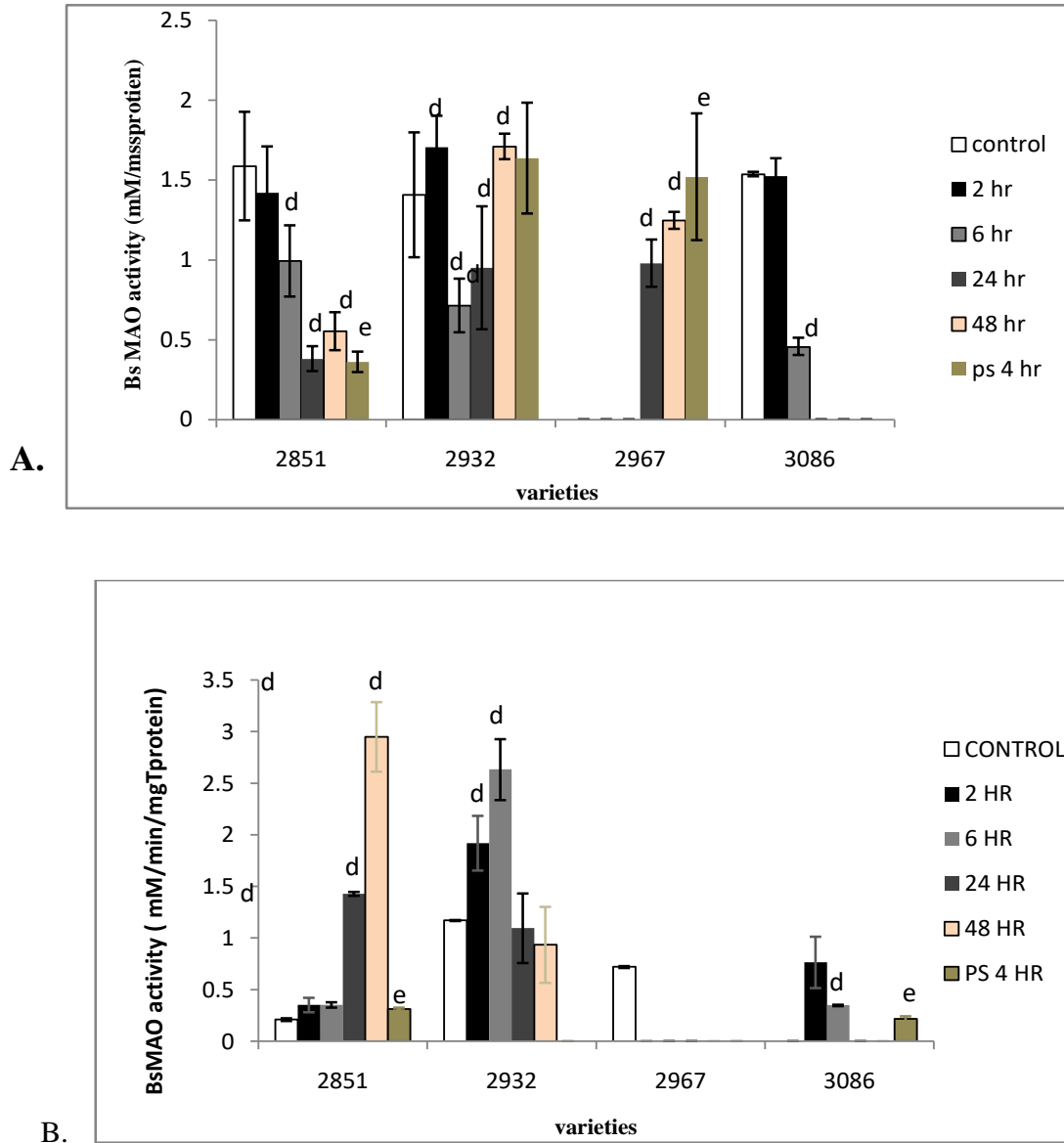


Figure 5 : MAO activity in Embryo (A) and Endosperm (B) in four diff cultivars under control and different drought stress treatment. symbol used : c- control, 2 hr- 2 hr drought stress, 6 hr- 6 hr drought stress, 24hr- 24 hr drought stress , 48 hr- 48 hr drought stress, PS – post stress. . Data shown are average \pm SE. ^d indicates significant difference between C v/s 2hrs-48hrs at $P \leq 0.05$. ^e indicates significant difference between 48hrs v/s PS 2hrs-PS 4hrs at $P \leq 0.05$.