THE EFFECT OF DROUGHT, HEAT AND COMBINED STRESS ON ANTIOXIDANT ENZYMES IN BREAD WHEAT GENOTYPES (*Triticum aestivum* L.)

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Abstract. Combined drought and high temperatures are mainly abiotic stress factors that often occur simultaneously in the field and represente an increasing risk to global wheat production. In addition, these environmental challenges lead to oxidative stress in plants through overproduction of reactive oxygen species (ROS). The effects of combined drought and heat on antioxidant enzyme activities were investigated in three wheat genotypes (*Triticum aestivum* L.). Two Algerian varieties, Ain Abid and Hidhab plus advanced line from CIMMYT, that called in this study V6 genotype, were compared under single and combined (HS x D) stresses of drought (30% field capacity, D) and heat stress (day/night = 23/36 °C, HS) during 7 days. Results showed that, heat stress (HS), drought (D) or combined of HS x D severely reduces growth, biomass (dry matter), with greater effect observed in response to HS x D by -58.38% as mean reduction for all genotypes. However, V6 genotype showed lower increase in malondialdehyde (MDA) concentration in response to single drought and heat stress that is closely associated with higher activity of CAT under drought (+304.79%) and APX activity under heat stress (+179.23%). While under combined of HS x D only V6 genotype showed efficient up-regulation of APX activity (+288.27%) that is followed by no significant oxidative damage (MDA). In contrast, Hidhab variety revealed inefficient increased of APX in response to combined of HS x D that is demonstrated by higher significant increase of MDA concentration. Similarly, in response to single drought (D), Ain Abid variety had inefficient up-regulation of CAT and the overproduction of peroxides might lead to membrane dysfunction that is a consequence of hydrogen peroxide accumulation due to loss in APX activity (-8.5%). Thus, our results suggest that tolerance of V6 genotype to drought, heat stress or their combined effect is strongly related with efficient modulation between antioxidant enzyme activities, less MDA concentration and increased CAT and APX activities to scavenge hydrogen peroxide in the plant cell.

Keywords: wheat genotype (*Triticum aestivum* L); drought; heat; oxidative stress and antioxidant enzymes.

INTRODUCTION

Wheat production in Algeria is mainly practiced in semi-arid areas, as a consequence, grain yield remains very low with annual production of bread wheat 9.52 million quintals and average yield 16.3 q^{-1} ha [11]. Moreover, cereal crops in Algeria suffer additional abiotic stresses such as winter-spring cold (due to altitude) and terminal drought (because of close proximity to the Sahara desert). The low rainfall and high temperatures are the serious threat to its low yield in arid and semi-arid areas [12].

During development cycle, wheat plants are subjected to various environmental stresses such as drought, cold, high temperatures, high salinity, etc. responsible for extensive curtailing of crop productivity worldwide [1, 39]. Wheat is a major crop plant on which the effects of drought [7, 21, 34, 44] and heat [3, 18, 42, 43] stresses given alone have been widely investigated at the physiological, genetic, and molecular levels; whereas, there is a very limited understanding of the combined effect of these two stresses. Moreover, in the field different stresses often occur simultaneously, such as high temperatures and drought periods, especially in semi-arid or drought-stricken areas [1, 37]. Furthermore, the biochemical responses of plants to the interaction of drought and heat are notable and cannot be directly estimated from the responses of plants to each of the separate stresses [37]. In spite of their importance, studies regarding combined effects of drought and heat stress impacts on plants are relatively few [47].

On the other hand, as consequence of primary stress effect, secondary stress such as oxidative damage in plant cell leads to a larger escape of electrons towards oxygen molecular during photosynthesis and metabolism processes, which enhances reactive oxygen species (ROS) generation such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) [9]. Thus, metabolic imbalances produced by changes in environmental conditions promote the over accumulation of ROS [50]. Interestingly, while ROS, such as H_2O_2, are considered important signal transduction molecules [14, 35], they are also toxic, causing extensive cellular damage and inhibition of photosynthesis [16]. Moreover, abiotic stresses cause limit of CO_2 availability due to stomatal closure and, thus, decrease plant productivity [52]. To mitigate the oxidative damage initiated by ROS, the plants have developed antioxidant system including ROS-scavenging enzymes such as catalase (CAT) and ascorbate peroxide (APX). H_2O_2 is eliminated by APX and CAT activities [38]. APX reduces H_2O_2 using ascorbate as the electron donor [23].

Many authors have reported that the ability of plants to balance ROS production and scavenging is associated to a higher tolerance to different environmental stresses [8, 27, 33, 35]. However, little is known about effects of combined of drought and heat on the antioxidative machinery on wheat genotypes. In the present work, we compare the tolerance level of three wheat genotypes (*Triticum aestivum* L.) subjected to individual drought, heat stress and their combination. The effects of these

stresses were investigated on growth, dry matter (biomass), lipid peroxidation (MDA), total soluble proteins and antioxidant enzymes. To achieve this, oxidative metabolism and some antioxidant enzyme activities were studies in three wheat genotypes (Ain Abid, Hidhab varieties and V6 genotype) with different ability to cope with this combined stress.

MATERIALS AND METHODS

Plant material and growth conditions

Pot experiment was conducted at National Institute of Agronomic Research of Algeria (INRAA), Laboratory of Plant Physiology, Biotechnology and Plant Breeding Division, Baraki, Algeria. Three wheat genotypes (*Triticum aestivum* L.) were grown in controlled environment greenhouse at optimum temperature (day/night, 23/14 °C) and relative humidity ranged from 65% to 75%. Two local varieties (Hidhab and Ain Abid) from Algeria were delivered by National Institute of Agronomic Research of Algeria (INRAA) and the third genotype coded as V6 in this study with followed pedigree (ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC) was from International Maize and Wheat Improvement Center (CIMMYT), this genotype is among the best advanced lines selected from national wheat breeding program and also evaluated in previous study [12].

Twelve seeds were hand sown on 20 cm pots containing soil of experimental station of Baraki with a clay texture plus sand mixed in 1:1 ratio. The pots watered to field capacity to facilitate germination, after a week only 10 plants were left per pot. At three leaf stage, 10 seedlings of each genotype were subjected to combination of two water regimes and heat stress. Water regime corresponded to (i) control (water applied 100% of container capacity throughout the experiment) and (ii) drought (D, water applied at 30% of container capacity), heat stress was applied by exposing plants to high nighttime temperature (day / night = 23/36 °C) during 7 days and the plants were kept in growth chamber under 16-h light / 8-h darkness.

Split-split-plot design was used to accommodate three-ways factorial experiment, with heat stress, water regime and genotype. Three single pot replicates per factorial combination were used, totaling 54 pots. All plants were growing in absence of stress until the beginning of three leaf stage. The water regime was imposed progressively over one week by decreasing irrigation and then heat stress treatment was applied by keeping the plants in growth chamber under a photoperiod 16-h light / 8-h darkness, heat stress (HS) treatment corresponding to group of plants which were treated at 23/36 °C (day / night) during 7 days.

Once water regime treatments (control and drought) were fully established at three leaf stage then a group of plants was grown under combination stress (heat stress x drought) during 7 days at 23/36 °C (day / night), samples were harvested after 24 days of experiment. Thus, a total of four treatments were study: (i) control, full irrigation (ii) Heat stress (HS), (iii) Drought (D) at 30 % of container capacity, (iv) combination of HS x D.

Plant growth

Plant samples were taken from every pot to monitor plant growth throughout the experiment. Shoot part of plants were cut it and then dried in an oven at 80 °C for 48 h and weighed for dry matter.

Total soluble protein content

Fresh leaves samples (0.1 g) were grinded with 1 mL of buffer (0.1 M Tris-HCl, 10 % sucrose and 0.05 % β-mercaptoethanol, pH = 8.1). After centrifugation at 10 000 g for 5 minutes, the supernatant is analyzed for measuring total soluble protein contents by spectrophotometer at 595 nm according to the method of Bradford [15] using bovine serum albumin (BSA) as a protein standard. All the steps of protein extraction were performed at 4 °C.

Assay of antioxidant enzyme activities

The enzymatic activities were measured by spectrophotometry, catalase (CAT) and ascorbate peroxidase (APX) activities were measured on the same extracts of total proteins. All the assays of the enzyme activities were performed at 4 °C.

Catalase activity (CAT)

Total catalase (EC 1.11.1.6) activity was measured by spectrophotometry at 240 nm using method of Anderson [5]. Declining in absorbance corresponded to hydrogen peroxide H_2O_2 (ε = 36 M^{-1} cm^{-1}) consumed by catalase enzyme, 50 µl of enzyme extract was mixed with 1.5 ml of 50 mM potassium phosphate buffer (pH = 7) and 12 µl of 6 % H_2O_2 was added to initiate the reaction.

Ascorbate peroxidase activity (APX)

APX (EC 1.11.1.11) activity was measured according to Nakano and Asada [40]. The assay depends on the decrease in absorbance at 290 nm as ascorbate is oxidized. The concentration of oxidized ascorbate was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX was defined as 1 µmol ascorbate oxidized per minute.

Lipid peroxidation

The level of lipid peroxidation in samples was determined in terms of thiobarbituric acid reactive substances (TBARS) according to the method of Rao and Sresty [45] from leaf samples.

Leaf samples (0.1 g) were homogenized in 1.5 ml of 1 % trichloroacitic acid (TCA) and centrifuged at 12 000 g for 15 min. Then, 500 µl of the supernatant was mixed with 1 ml of 0.5 % thiobarbituric acid, heated at 95 °C for 30 min and then was quickly cooled down on ice. Absorbance was measured with a spectrophotometer at 532 and measurement was corrected at 600 nm for unspecific turbidity [53]. The MDA content was calculated by using the extinction

coefficient of (ε = 155 $mM^{-1}cm^{-1}$) and expressed as µmol of MDA g^{-1} fresh weight.

Statistical analysis

Data were subjected to factorial analyses of variance (ANOVA) with three factors (heat stress, water regime and genotypes) using the GenStat Discovery software package. Then, the differences between the means were compared by Fisher's Least-significant Difference test (LSD) at a probability level of 95%. Significance levels were expressed as p = 0.05 and data were significant when $p < 0.05$.

RESULTS

Effect of treatments on growth

Heat stress, drought and their interactive effects caused a significant ($P < 0.05$*) decrease in dry matter of shoots in wheat (Fig. 1). Drought treatment applied had more drastic and significant reduction in dry matter than heat stress treatment. The shoot dry matter of the three genotypes were (0.668 g) for control, (0.492 g) for HS, (0.297 g) for D and (0.278 g) for their combination HS x D, indicating that the effect of the drought treatment and combined stress factors had a more severe effect on dry matter that the heat treatment. The relative decrease of shoot weights compared with the control of the three genotypes were -26.35% for HS, -55.54% for D and -58.38% for HS x D. Thus growth was less limited under drought (D) than combined stresses but statistically there were no significant difference between them.

The dry matter of all genotypes decreased significantly ($P < 0.05$) under all treatments imposed. While under heat stress V6 genotype showed no significant decrease in dry matter (Fig. 2), where the effect of heat stress was (-18.84%). On the other hand, V6 genotype still recorded the lowest reduction in dry matter under drought with - 43.80% of the control, in contrary, Hidhab variety was exhibited the highest reductions for dry matter under individual drought (-62.96%) compared to control. However, in response to HS x D, V6 genotype was recorded the highest lost in dry matter with rate of -60.44% of the control, whereas the lowest reduction was in Hidhab variety with a rate of -55.34%.

Total soluble protein content

Data presented in figure 3 showed that individual heat stress (HS) had significant effect on soluble protein content than drought conditions (D). The maximum significant decrease in soluble protein was observed in response to individual heat stress (-42.32%) HS and combination of heat and drought (-26.80%) HS x D.

Soluble protein content was examined in leaves of three wheat genotypes under individual and combined stresses (Fig. 4). Drought caused no significant decrease in soluble protein except for Hidhab variety where there was slight increase (7.14 to 7.25 $mg \bullet g^{-1}$ FW),

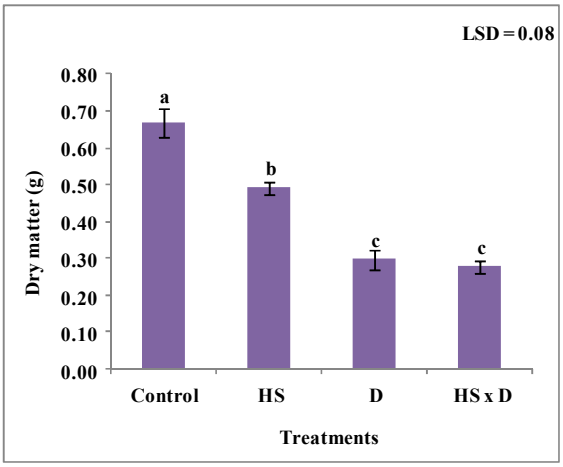

Figure 1. Mean dry matter of three wheat genotypes (*Triticum aestivum* L.) under heat stress (HS), drought (D) and combined stresses (HS x D). LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$).

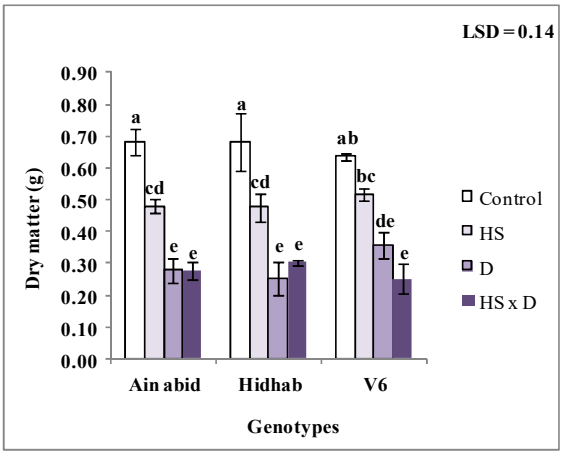

Figure 2. Dry matter of three wheat genotypes (*Triticum aestivum* L.) under heat stress (HS), drought (D) and combination effects of drought and heat stress (HS x D). LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$*).

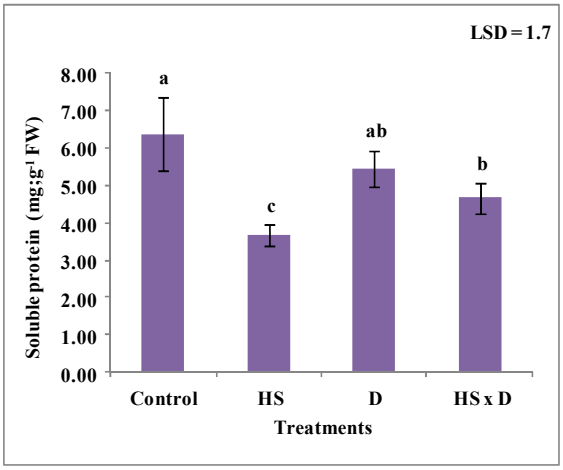

Figure 3. Effect of heat stress (HS), drought (D) and their combination on total soluble protein content in leaves of three wheat genotypes (*Triticum aestivum* L.). LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$*).

whereas heat stress induced decrease in protein pool level, this effect was more significant in Ain Abid and V6 genotype with reduction rates (-46.32% and -47.46% respectively).

Besides that, all genotypes exhibited no significant decreased of soluble protein content in response to combined stress factors (HS x D). The highest reduction rate was recorded in Ain Abid variety with -32.63% and the lowest rate of reduction was -19.89% in Hidhab variety (Fig. 4).

Antioxidant enzyme activities

To avoid potential damage of abiotic stress caused by reactive oxygen species (ROS) to cellular components, enzymatic and non-enzymatic antioxidants plays sustainable equilibrium between the production and detoxification of ROS. In this regard, we examined the activities of catalase (CAT) and ascorbate peroxidase (APX) from wheat leaves subjected to heat stress, drought and combined stresses.

Catalase activity (CAT)

As shown in figure 5, catalase activity increased significantly in response to heat stress (HS) and drought (D). However, the magnitude of the increase was much larger in drought (+373.18%) of control than in heat treatment (+171.99%) of control. While no significant increase of catalase was observed under combined stresses. We observe a significant efficiency of catalase activity under drought in comparison to the heat stress (Fig. 5).

The effect of heat stress, drought and their combination on catalase activity for all genotypes tested is reported in figure 6. The expression of catalase activity has a basal level at control and then its expression increased under HS for all genotypes the maximum increased was in Hidhab variety with a rate of +131.77% of control.

In exception, catalase activity was markedly induced under single drought (Fig. 6). In Ain Abid and V6 genotype, accumulation of catalase revealed a remarkable up-regulation under individual drought (D) with greater extent in Ain Abid variety (+2053.4%) and then followed by V6 genotype (+304.79%) based on means data values. Whereas under combined effect of stresses there was no significant difference of catalase activity for all genotypes.

Ascorbate peroxidase activity (APX)

Independently of genotype effect, APX activity increased in response to drought (+52.59%), whereas a significant increment in APX activity were observed in response to HS (+89.64%) and combination of HS x D (+165.60%) with respect to control (Fig. 7). Our data showed that, APX activity is more efficient under combined stress (HS x D) than single stresses.

As showed in figure 8, individual drought significantly enhanced APX activity in Hidhab variety (+214.93%) than in V6 genotype (+28.20%), whereas for Ain Abid variety it decreased by -8.5%. Moreover,

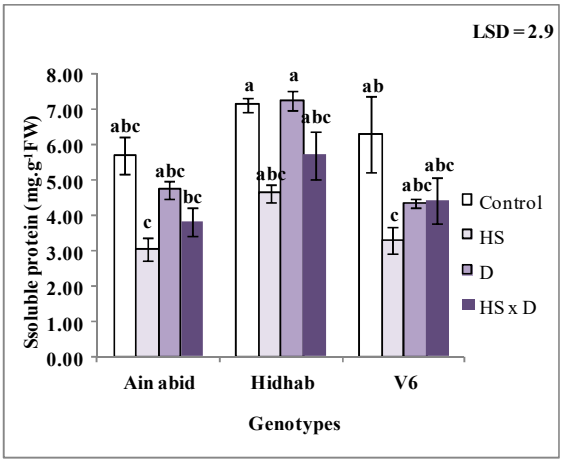

Figure 4. Total soluble protein content in leaves for three wheat genotypes (*Triticum aestivum* L.) under heat stress, drought and combined stresses. LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$).

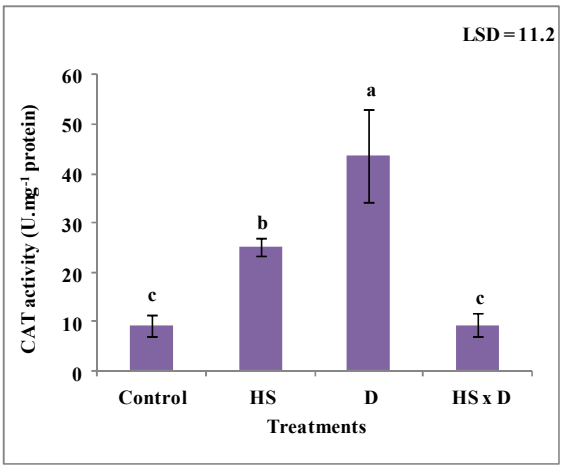

Figure 5. Mean change in catalase (CAT) activity of three wheat genotypes (*Triticum aestivum* L.) subjected to heat stress (HS), drought (D) and combined stresses (HS x D). LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$).

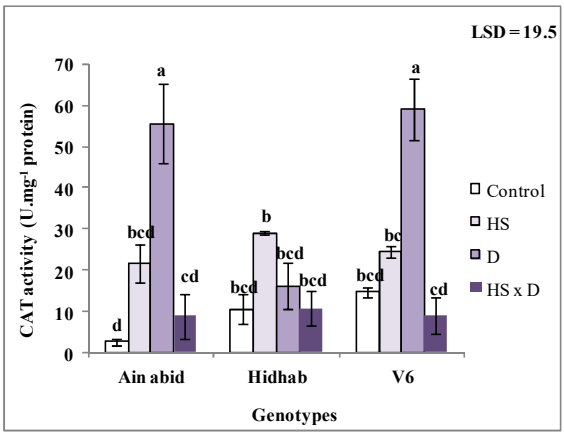

Figure 6. Catalase activity for three wheat genotypes (*Triticum aestivum* L.) under heat stress, drought and combined stresses. LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different ($P < 0.05$).

heat stress increased significantly APX activity only in V6 genotype (+179.23%). While in response to combined stress (HS x D), V6 genotype and Hidhab variety increased significantly their APX activity with rates of +288.27% and +273.61% respectively. However, we observed that expression of APX activity was up-regulated under HS and especially in response to combination of HS x D in V6 genotype. Whereas single drought and combination of HS x D increased antioxidant activity of APX in Hidhab variety.

Lipid peroxidation assay

The levels of lipid peroxidation produced in the leaves under heat stress, drought and combined stresses were determined using the thiobarbituric acid (TBA) test. This test calculates malondialdehyde (MDA) as a final product of lipid peroxidation process [30].

The response of MDA content to the applied stresses varied significantly (Fig. 9). Oxidative damage expressed as MDA accumulation in response to individual heat stress (HS) showed slightly increase (+0.25%), but we did not observe a significant difference compared to the control. However, MDA concentration in leaves was accumulated significantly in response to drought (+42.82%) and more prominently under combination of HS x D (+57.92%).

In drought conditions, Ain Abid and Hidhab varieties increased significantly MDA content with rates of +74.23% and +68.53% respectively (Fig. 10). On the other hand, the amount of increased MDA in V6 genotype was not significant indicating a low oxidative damage and therefore more stability of cellular membranes against environment stresses. Moreover, we observe slight increase of MDA content in V6 genotype (+13.95%) and Hidhab variety (+2.79%) in response to HS, whereas it was more in Ain Abid variety by (+18.48%).

The malondialdehyde content tends to show greater accumulation under combined stresses (Fig. 10) for all

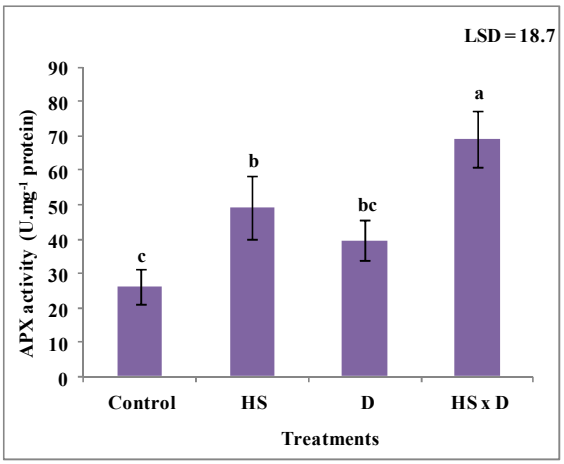

Figure 7. Mean change in ascorbate peroxidase (APX) activity of three wheat genotypes (*Triticum aestivum* L.) subjected to heat stress (HS), drought (D) and combination of heat and drought (HS x D). Means of 3 replication ± SE. Bars with different letters are significantly different (*P < 0.05*).

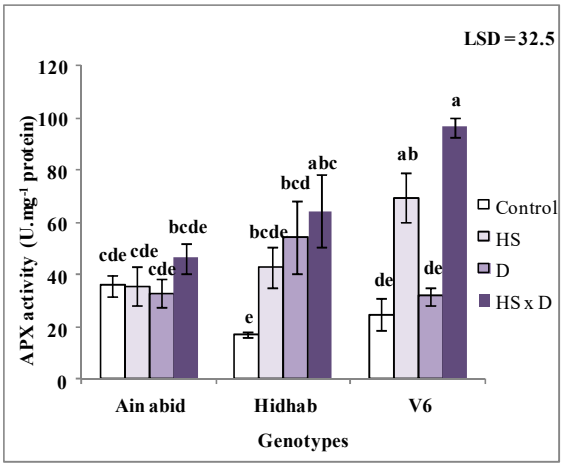

Figure 8. APX activity for three wheat genotypes (*Triticum aestivum* L.) under drought, heat stress and combined stresses. LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different (*P < 0.05*).

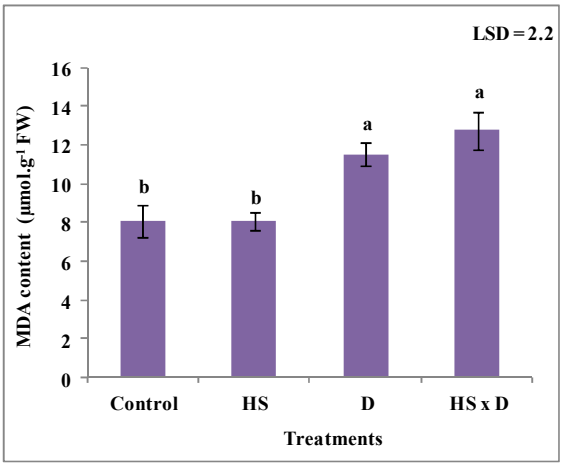

Figure 9. Mean malondialdéhyde (MDA) accumulation of three wheat genotypes (*Triticum aestivum* L.) subjected to heat stress (HS), drought (D) and combined stresses (HS x D). LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different (*P < 0.05*).

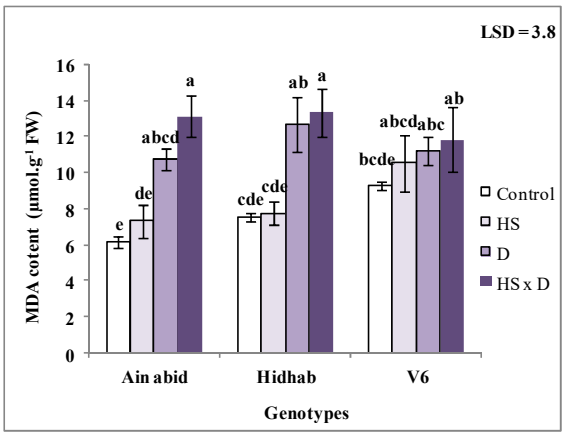

Figure 10. Malondialdehyde (MDA) accumulation in leaves of three wheat genotypes (*Triticum aestivum* L.) subjected to drought, heat stress and combined stress factors. LSD = Least Significant Difference. Means of 3 replication ± SE, bars with different letters are significantly different (P < 0.05).

genotypes. However, we recorded significant increment of MDA content in Ain Abid and Hidhab varieties in response to HS x D by +112.64% and +77.03% respectively, while this effect was low and no significant for V6 genotype with rate of +27.89% compared to control (Fig. 10).

DISCUSSION

Among the most limiting environmental conditions, heat stress, drought or different combinations of environmental challenges, induce metabolic imbalances that can cause an oxidative stress in plant cells. In addition, these stresses are counted as an important threat to plant development and their productivity. These abiotic stresses results in the generation and accumulation of ROS, promoting oxidation of cellular components, hindering metabolic activities and affecting organelle integrity [50]. In the present work, the antioxidant system of three wheat genotypes (*Triticum aestivum* L.) with different ability to tolerate the individual and combination of heat and drought was performed, to assess the variation response of some enzymatic antioxidant systems such as catalase (CAT) and ascorbate peroxidase (APX) to this abiotic stress.

Growth was significantly restricted for all genotypes under heat stress and drought but it was more drastic reduction in response to combination stresses (Fig. 1). The low availability of soil moisture results in decreased nutrient transport towards roots, according to Kramer and Boyer [31], drought conditions impaired active transport and membrane permeability and reduction of transpiration rate which decreased the nutrient absorption efficiency of roots [20]. Thus, as results of drought stress and nutrient imbalance, plant growth is reduced [28]. In addition, reduction in dry matter could be a result of restricted hydrolysis of carbohydrates reserves and their translocation to shoots. Ours results showed that, plant growth (through dry matter) reduced in all genotypes when they subjected to drought, heat stress and combined HS x D stresses (Fig. 2). However, biomass accumulation (dry matter) was less affected in V6 genotype than Ain abid and Hidhab varieties. This result suggesting that V6 genotype has ability to maintain quite satisfactorily its biomass in harsh environment, this proves well stability in photosynthetic activity under stresses environment, which might have resulted in maintaining dry matter of this genotype compared to others tested in this study.

The significant reduction of total soluble protein was observed in response to combined heat stress and drought as well as under individual heat stress which was more drastic (fig. 3). This change in protein expression have been reported for many species as results of plant subjected to drought, salinity, heat stress and other abiotic stress [2, 25, 29, 32]. The reduction in total protein content in plants under drought was due to protein synthesis inhibition, increase of proteolysis process or decrease in amino acid content [19]. On the other hand, heat stress and combination of HS x D reduced more the soluble protein this could be due to protein synthesis inhibition and proteolysis which occurs in same time, moreover, nitrate reductase involved in protein synthesis is the most altered enzyme under abiotic stress [49].

Heat stress, drought and their combined effect led to oxidative stress as result of this stress lipid peroxidation [13]. Malondialdehyde (MDA) is ultimate product of membrane peroxidation, and its content is linked to damage extent of reactive oxygen species [10, 48, 51]. The MDA is regarded as marker for assessing lipid peroxidation or damage to cytoplasmic and organelles membranes [17]. Our study showed that MDA content in leaves increased under drought, heat stress and more when stresses are combined. Indeed, Lipid peroxidation (MDA) in leaves of three genotypes was correlated with growth inhibition (dry matter) under heat stress, drought and their combination.

Our results showed that, MDA content was substantially higher in Hidhab and Ain Abid varieties under combined stress (Fig. 10). Whereas the level of lipid peroxidation (MDA) was lower in V6 genotype in response to combined stress (HS x D), this probably due to the higher up-regulation of APX activity that participated in H_2O_2 detoxification. Moreover, MDA content did not change significantly by heat stress and drought alone, this indicating V6 genotype is better protected against oxidative damage under combination of heat stress and drought.

Otherwise, enzymatic antioxidant systems such as catalase (CAT) and Ascorbate peroxidase (APX) have an influential role in plant defenses against ROS [36]. From our finding, oxidative damage estimated by MDA accumulation was also lower in leaves of V6 genotype in response to individual drought and heat stress which is directly linked with higher expression level of catalase activity under drought and APX activity under heat stress (Fig. 6, 8), suggesting that, the ability of CAT to scavenge H_2O_2 in cytosol and peroxisome [26, 41] and APX dismutase H_2O_2 in chloroplast, mitochondrion, apoplast, peroxisome and cytosol [9, 46] using ascorbate as the electron donor [22]. On the other hand, in response to single drought (D), Ain Abid variety had inefficient up-regulation of CAT and the overproduction of hydrogen peroxide and the loss in APX activity (-8.5%) have lead to membrane dysfunction[6], for that, we have recorded higher level of MDA content.

However, the increased of CAT and APX activities of V6 genotype in both single stresses could be related to an active and efficient antioxidant response that might be involved in maintaining a lower MDA concentration [24] especially under the combination of drought and heat, and therefore helping wheat plants to cope with the combined stresses.

In conclusion, our study results showed a varied response in bread wheat genotypes (*Triticum aestivum* L.) to combination of heat and drought. This variation

was revealed by the assessment of the impact of heat stress, drought and their combined action on growth, biomass, MDA concentration and antioxidant enzymes of three wheat genotypes indicating that, V6 genotype was more adapted under stress. The tolerance of V6 genotype to heat stress (HS) and drought (D) is firmly linked to higher capacity of CAT activity, therefore a less MDA content under drought (Fig. 6) and efficient activation of APX under heat stress (HS), as well as under combination of HS x D (Fig. 8). In contrast, Hidhab variety in response to HS x D and individual drought (D) showed low CAT activity (Fig. 6) and a lack of APX activity increase (Fig. 8), this could be partially responsible of its increased oxidative damage under combination of HS x D and single drought. The severe effect of combined HS x D stress on growth which is followed by significant increase of APX activity might be explained by synergistic effect of both stresses. Heat stress showed no effect on MDA concentration while drought caused significant effect. Thus, in this case combined effect of HS x D was the additive type. On the other hand, for CAT activity, it seems that there was antagonist effect in response to combined stress. Thus, drought and heat stresses might have synergistic, additive or antagonist effects according to studied parameters.

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