EXOGENOUS HYDROGEN PEROXIDE ALLEVIATES WATER STRESS-INDUCED PHYSI-OLOGICAL AND BIOCHEMICAL CHANGES IN DURUM WHEAT (*TRITICUM DURUM* DESF.)

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ABSTRACT.*-* Water scarcity threatens crops, in particular, durum wheat (*Triticum durum* Desf.), in the world's drought regions. As the water-stress wheat cultivars in the middle east of Algeria are poorly investigated, the present study was, therefore devoted to exploring the effect of water deficiency and, the possible attenuative role of exogenous hydrogen peroxide (H₂O₂) in Sémito, a commonly cultivated durum wheat (*Triticum durum* Desf.) variety in the middle eastern regions of Algeria. Here, Water-stressed durum wheat seeds received sufficient water for 48 hours to allow for uniform seed germination and then were subjected to cease the watering phase. The attenuation of water stress severity was examined in water-stressed wheat seeds treated with two different concentrations of H_2O_2 (20 and 50 mM). Water stress significantly reduced mean root number (MRN), mean root length (MRL), and germination percentage (G %), in addition to a marked decline in total protein content, and increased level of proline content and catalase activity compared to control plants. Moreover, H_2O_2 co-treatment enhanced the catalase activity, and promoted the accumulation of proline and protein contents, contributing to osmotic adjustment under water stress conditions. Our findings suggest that exogenous H_2O_2 application ameliorates water stress-induced physiological and biochemical changes in durum wheat, highlighting its potential as a promising strategy to enhance drought tolerance in this economically important crop species.

INTRODUCTION

Durum wheat (*Triticum durum* Desf*.*) is the main nutrient cereal-based food in the Mediterranean countries (Bouthiba *et al.* 2008, Fellah *et al.* 2018), and numerous farmers (Durante *et al.* 2012, Saini *et al.* 2023), however, its yield and productivity can be adversely affected by several environmental stressors including water stress (Kettani *et al.* 2023; Soorninia *et al.* 2023). Water stress refers to the condition in which plants suffer from water or inadequate water availability for optimal growth and physiological processes(Zhu *et al.* 2023). Hence, water stress of water deficiency, impacting crop production and food security, is a worldwide agricultural concern (Simbeye *et al.* 2023). As a result, there is a need to underscore the urgency of exploring innovative strategies that enhance plant resilience to limited water availability. In recent years, researchers have begun to investigate the potential of exogenous hydrogen peroxide $(\mathrm{H}_{2}\mathrm{O}_{2})$ in improving the impact of water stress on various cereal crops(Iqbal *et al.* 2023, Basal *et al.* 2024). Hydrogen peroxide, a reactive oxygen species, has received great scientific

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attention as a signaling molecule involved in orchestrating a cascade of responses to abiotic stress in plants(Choudhury *et al.* 2013, Ul Islam *et al.* 2023). The exogenous application of hydrogen peroxide proved its efficiency in inducing stress tolerance and bolstering antioxidant defense mechanisms in diverse plant species(Choudhury *et al.* 2013, Wang *et al.* 2024). However, the specific implications of exogenous hydrogen peroxide on durum wheat under water stress conditions, particularly within the context of Algerian agricultural systems, remain a significant research gap. This study, therefore, aims to bridge this gap by exploring the beneficial role of exogenous hydrogen peroxide application on water scarcity-induced physiological and biochemical alterations in a commonly cultivated durum wheat (*Triticum durum* Desf.) variety, Sémito, in the Middle Eastern regions of Algeria. Our study seeks to provide novel insights into the adaptive strategies employed by durum wheat under water stress conditions.

MATERIALS AND METHODS

Biological material

In this study, a durum wheat variety named Semito (*Triticum durum* Desf.) of the Poaceae family was obtained from the Middle East region of Algeria (the Interprofessional Cereal Office (AIPCO), Algeria).

Chemical materials

Hydrogen peroxide solution (H_2O_2) was purchased from Merck Co. (Darmstadt, Germany). All other chemicals used in this study were of Analytical Reagent grade.

Methods

Durum wheat seeds were placed per 10 into Petri dishes containing filter paper, and treated with 5ml of prepared solutions during the germination phase. The wheat seeds were divided into a control group that received distilled water, two groups treated respectively with 20 and 50mM of H_2O_2 , a water stress group that underwent ceasing watering from the 2nd day of cultivation, and two groups that underwent water stress condition and received 20mM and 50mMH₂O₂.

Determination of morphophysiological parameters

The germination percentage was determined in the germination stage (48 hours after the seeds were initially placed under germination conditions), while the morphophysiological parameters of durum wheat, including the mean root number (MRN), and the mean root length (MRL) were determined after 7 days 1 week after sowing. The control wheat seeds received distilled water, which was replaced by tap water to ensure the normal growth of the young wheat plant with only $\rm H_2O_2$ treatment, or $\rm H_2O_2$ co-treatment with water-stress conditions. The germination percentage was determined based on the following formula:

$$
Germanation\,percentage(G\,\%) = \frac{X}{Y} \times 100
$$

Where "**X"** is the number of germinated seeds, and "**Y"** is the number of total seeds.

Determination of biochemical parameters

An aliquot of 100 μl solution containing dried wheat roots was used to determine the total protein content (Bradford 1976), and proline content (Al-Khayri & Al-Bahrany 2004), in the presence of 4 % diluted acetic acid solution 528 nm. Catalase activity in the sampled wheat plant was determined as described elsewhere (Kolupaev *et al.* 2005), where the enzymatic activity was measured based on the rate of absorbance decrease at 240 nm of a solution containing 30 mM H_2O_2 in 50 mM potassium buffer (pH 7.0). These parameters were determined using a double-beam UV-Vis Spectrophotometer.

Statistical analysis

Data were presented as mean \pm standard deviation (SD). Comparison between groups was performed using oneway ANOVA followed by Tukey's multiple comparisons test, utilizing Prism software (Version 5, Windows). A significance level of $p < 0.05$ was considered statistically significant.

(2.46 \pm 0.85), and not significantly in 50mM H₂O₂treated plants (3.83 \pm 0.31) and 50 mM H₂O₂treated water stressed plants (3.56 ± 0.54) when compared with control plants (4.23 ± 0.4) .

Fig. 1.- Variation of mean length roots of wheat subjected to H_2O_2 and/or water stress (WS) treatment. Data are represented as the mean \pm SD (n = 10). ns (not significant) $p > 0.05$, *p < 0.05, and **p < 0.01 are statistically significant versus control and water stress (WS) treatment.

Further, MRL increased significantly in water-stressed plants treated with 20 mM H_2O_2 (p < 0.01) $(6.39 \pm 0.44 \text{vs} 2.46 \pm 0.85)$ and 50 mM H₂O₂ (p < 0.05) $(5.39 \pm 0.44 \text{ vs } 2.46 \pm 0.85)$ compared with plants underwent water stress. In Fig. 2, the root mean number (RMN) increased significantly in wheat treated with 20 mM H₂O₂(p < 0.001) (6.89 \pm 0.69), not significantly in 50mM H₂O₂ (4.03 \pm 0.62), and 20 mM H₂O₂-treated water-stressed wheat roots (5.13 \pm 0.67), but decreased significantly in water stress condition ($p < 0.05$) (2.76 \pm 0.49), and 50 mM H₂O₂-treated water-stressed wheat roots ($p < 0.05$) (2.70 \pm 0.73) compared with controls (4.43 \pm 0.60). While, the RMN increased significantly ($p < 0.05$) and not significantly, respectively in water stressed wheat roots treated with 20mM H₂O₂ (5.13 \pm 0.67 vs 2.76 \pm 0.49), and 50mM H₂O₂ (2.70 \pm 0.73 vs 2.76 \pm 0.49) when compared with the water-stressed plant. Moreover, the germination percentage revealed a significant and non-significant increase in 20 mM H₂O₂ ($p < 0.01$) (47.73 \pm 2.49), and 20 mM H₂O₂-treated water-stressed seeds (72.56 \pm 6.749) respectively, along with a significant (p < 0.05) and a non-significant decrease, respectively in water-stressed seeds (31.5 ± 7.5) , and 50 mM H₂O₂-treated water-stressed seeds (42.13 \pm 5.53) as compared with controls (47.73 \pm 2.4). In the combined treatments compared with water-stress conditioned seeds, the germination percentage revealed a non-significant increase in 20 mM of H₂O₂-treated stressed wheat seeds (52.2 \pm 9.18 vs 47.73 \pm 2.40), and a non-significant decrease in 50 mM of H₂O₂-treated stressed wheat seeds $(42.13 \pm 5.53 \text{ vs } 47.73 \pm 2.40)$ (Fig. 3).

Fig. 2.- Variation of root mean number of wheat subjected to H_2O_2 and/or water stress (WS) treatment. Data are represented as the mean \pm SD (n = 10).

ns (not significant) $p > 0.05$, *p < 0.05, and **p < 0.01 are statistically significant versus control and water stress (WS) treatment.

Fig. 3.- Variation of germination percentage of wheat seeds subjected to H_2O_2 and/or water stress (WS) treatment. Data are represented as the mean \pm SD (n = 10). ns (not significant) $p > 0.05$, *p < 0.05, and **p < 0.01 are statistically significant versus control and water stress (WS) treatment.

Biochemical parameters

Protein content decreased significantly in the water-stressed wheat plant ($p < 0.01$) (0.27 \pm 0.06), and not significantly in 50 mM $H_2O_2(0.4 \pm 0.06)$ and 50 mM H_2O_2 -treated water-stressed plants (0.38 ± 0.04) , but it increased significantly in 20 mM H₂O₂ treatment (p < 0.001) (0.71 \pm 0.04), and not significantly in 20 mM H_2O_2 -treated water-stressed plants (0.56 \pm 0.05) compared with controls (0.46 \pm 0.03). This parameter increased significantly in water-stressed plants treated with 20 mM H₂O₂ (p < 0.01) (0.56 \pm 0.05) and 50 mM H₂O₂ (p < 0.05) when compared with water-stress plants (0.27 \pm 0.06) (Fig. 4).

Fig. 4.- Variation of protein content in wheat subjected to H_2O_2 and/or water stress(WS) treatment.

Data are represented as the mean \pm SD (n = 10). ns (not significant) p > 0.05, *p < 0.05, **p < 0.01 ***p < 0.001 are statistically significant versus control and water stress (WS) treatment.

Proline content increased significantly in wheat plants underwent water stress ($p \le 0.01$) (7.1 \pm 0.62), water-stressed plants treated with 20 mM $H_2O_2(p < 0.001)$ (8.14 ± 0.64), and 50 mM $H_2O_2(p < 0.0001)$ (9.59 ± 1.06) , and not significantly in 20 mM H₂O₂ (4.71 \pm 1.14) and 50 mM H2O2 (5.56 \pm 1.13) treated plants compared with control plants (4.11 ± 0.48) . Similarly, proline content increased but not significantly in H_2O_2 -treated water-stressed plants when compared with stress-conditioned plants (Fig. 5).

Fig. 5.- Variation of proline content in wheat subjected to H_2O_2 and/or water stress (WS) treatment. Data are represented as the mean \pm SD (n = 10).

ns (not significant) $p > 0.05$, $\frac{1}{p} < 0.05$, $\frac{1}{p} < 0.01$, $\frac{1}{p} < 0.001$ and $\frac{1}{p} < 0.0001$ are statistically significant versus control and water stress (WS) treatment.

On the other hand, a significant increase in catalase activity was noticed in wheat plants subjected to water stress conditions ($p < 0.001$) (7.92 \pm 0.87), and water-stressed plants treated with 20 mM H₂O₂ $(p < 0.01)$ (7.54 ± 0.69) and 50 mM H₂O₂ ($p < 0.05$) (6.64 ± 0.51) as compared with control plants (4.85 ± 0.54) . Whilst, the enzymatic activity showed non-significant changes in plants treated only with 20 mM H_2O_2 (4.37 \pm 1.4) and 50 mM H_2O_2 (4.91 \pm 1.17) compared with controls, and the combined treatments when compared with water-stressed plants (Fig. 6).

Fig. 6.- Variation of catalase activity in wheat subjected to H_2O_2 and/or water stress(WS) treatment.

Data are represented as the mean \pm SD (n = 10).

ns (not significant) $p > 0.05$, $\binom{*}{p} < 0.05$, $\binom{*}{p} < 0.01$, and $\binom{**}{p} < 0.001$ are statistically significant versus control and water stress (WS) treatment.

DISCUSSION

As water scarcity is a worldwide agricultural concern, various approaches are applied to enhance drought tolerance in diverse plant species and crop cultivars, encompassing the application of chemicals via different means (Bohnert & Jensen 1996, Jamil *et al*. 2015, Merewitz 2016). In this regard, our study was performed to examine H_2O_2 enhancing the tolerance of wheat variety, Semito, to water stress. In this study, the obtained morphophysiological results of wheat showed a marked increase in the mean root length (MRL) and root mean number (MRL) in 20mM H_2O_2 , and a slight decrease in 50mM H_2O_2 treatment as compared with the control. Hence, the low concentration of 20mM H_2O_2 seems to have no toxic effect, but likely effective beneficial effect on root growth. It was reported that a non-toxic concentration of H_2O_2 can improve the growth parameters of plants subjected to various stress conditions (Wahid *et al.* 2007). Also, H_2O_2 -induced growth stimulation of plant seeds has been previously reported in some plant species, including *Zinnia elegans*, *Panicum virgatum*, and *Andropogon gerardii* (Ching 1959, Ogawa & Iwabuchi 2001, Sarath *et al.* 2007). In fact, H₂O₂ plays a dual role as a messenger involved in several cellular mechanisms at low concentrations, and as an oxidative stress inducer resulting in cell death and damage at high concentrations (Miyake & Asada 1996). However, the water stress condition decreased the root mean number and the mean root length of wheat, which is somehow likely due to stress-induced growth inhibition. This result is agreed with that previously reported (Soltani *et al.* 2006). In this study, the supplementation of H_2O_2 to water-stressed wheat improved the wheat tolerance against water stress conditions, and this has been previously proved (Wahid *et al.* 2007). Further, results revealed that $20 \text{mM H}_2\text{O}_2$ stimulated seed germination, and this has been confirmed in a previous study investigating the beneficial effect of exogenous H_2O_2 on the germination of wheat seeds, and some other seed plants, including [Hordeum vulgare](https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2021.682439) and Arabidopsis thaliana through the metabolism regulation of abscisic acid (ABA) and gibberellic acid (GA) (Liu *et al.* 2010), known as key hormones involved in regulation of dormancy and germination (Graeber *et al.* 2014). Nevertheless, water stress exposed wheat seeds markedly decreased the germination percentage. This result has been similarly reported in a previous study investigating the effect of water stress on

winter wheat (Mian & Nafziger 1994, Alvarado & Bradford 2002, Norsworthy & Oliveira 2009). The low germination percentage in water-stressed wheat is likely due to reason of that the water deficit impedes the enzymatic activity of some enzymes, in particular, amylase leads to the breakdown of stored nutrients needed for germination, and reduction of the energy available for the seed embryo, which slows or halts germination, in addition to the cellular structure damages and inhibition of DNA, RNA, and protein synthesis which are vital processes for germination and seedling development (Yu *et al.* 2016). Also, drought conditions increase the production of reactive oxygen species (ROS), which can cause oxidative damage to cellular structures, including DNA and proteins. In extreme cases, this damage can prevent germination entirely by killing the embryo (Cruz de Carvalho 2008). On the other hand, H_2O_2 treated water stressed wheat slightly stimulated germination, in accordance with some previous (Iqbal *et al.* 2018, dos Santos Guaraldo *et al.* 2023). This action can refer to the improvement of plant tolerance to abiotic stress through the activation of a series of mechanisms in response to stressors (Chen *et al.* 2021). In this study, protein content decreased slightly in $50 \text{mM H}_2\text{O}_2$ treatment, but markedly in waterstressed plants compared with the control. As reported (Rasheed *et al.* 2020, Siddiquy *et al.* 2023), several types of proteins whose structure enter into the constitution of plant tissues and play a crucial role in cell death and cell signaling pathways, involving mainly enzymes, hormones, and heat shock proteins). The decreased level of proteins in plants exposed to high H_2O_2 concentrations and/or stress conditions can be explained by the induction of oxidative stress associated with the generation of free radicals resulting in the oxidation of cell macromolecules, in particular, proteins (Kumar *et al.* 2023, Momeni *et al.* 2023). Interestingly, the supplementation of H_2O_2 significantly improved the level of protein content. Similar results have been reported in Arabidopsis (Fryer *et al.* 2003). Under stress conditions, plants activate various molecular mechanisms, including drought-responsive genes, signaling pathways, secondary messengers, and transcription factors (Mukherjee *et al.* 2023, Buragohain *et al.* 2024), and noteworthy hydrogen peroxide involved in cell signaling can serve as a second messenger in cellular signal transduction, and can lead to the activation of Mitogen-activated protein kinase kinases (MAPKs) in the case of the changes in the external environmental conditions (Jamshidi Goharrizi *et al.* 2023). Furthermore, proline content increased slightly and not significantly in the supplementation of H_2O_2 treatments, and significantly in wheat plants subjected to water stress and those received the combined treatments. Proline is an osmoprotectant that plays several roles in plants, including osmotic adjustment, signaling as well as ROS detoxification (Singh *et al.* 2015, Székely 2004, Zulfiqar *et al.* 2020). It reduces the harmful effects of ROS by stimulating the antioxidant defense mechanism through osmotic regulation and by protecting the integrity of cell membranes (Banu *et al.* 2009, Reddy *et al.* 2015). The increased level of proline in the supplementation of H_2O_2 treatment was reported in some previous studies (Liao *et al.* 2016, Liu *et al.* 2020, Singh *et al.* 2021). H₂O₂ was reported to act as a signaling molecule, activating pathways that lead to increased expression of enzymes involved in proline biosynthesis, such as pyrroline-5-carboxylate synthetase (P5CS) (Yang *et al.* 2009). Similarly, water deficit conditions caused a significant increase in proline content in wheat compared with control. This result was reported in previous studies investigating plant response to water and salinity stress, cooling, and temperature variations (Cramer *et al.* 2007, Szabados & Savouré 2010). Proline accumulation in cells leads to an increase in osmotic potential and greater water uptake capacity by roots and water saving in cells (Anita *et al.* 2018). What's more, catalase activity revealed no significant changes in H2 O2 treatments(Moskova *et al.* 2009). Whist, catalase activity increased significantly in water-stressed plants, and those supplemented with $\rm H_2O_2$, and this is in agreement with that previously reported (Sairam & Srivastava 2001). This enzymatic increase was less important in H_2O_2 -treated water-stressed plants

compared to plants subjected to water-stress conditions, suggesting thus the beneficial role of hydrogen peroxide in improving the antioxidant defense system against various stress conditions (Kocsy *et al.* 2001).

CONCLUSION

The application of hydrogen peroxide enhanced the activity of antioxidant enzymes compared to the control, which resulted in H_2O_2 scavenging efficiency. Data suggest that exogenous H_2O_2 could modulate the wheat defense response to water stress through the regulation of peroxide production.

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