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Nitric Oxide and Hydrogen Peroxide as Signalling Molecules for Better Growthand Yield of Wheat Plant Exposed to Water Deficiency

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Abstract

Two pot experiments were performed in randomized complete block design with four replications. Wheat grains primed with Sodium Nitroprusside (SNP) and Hydrogen peroxide H2O2 (0, 50 and 100 µM) as well as water deficiency were the studied factors. Water deficiency significantly decreased plant growth indices, relative water content (RWC%), grain yield concomitant with decreasing leaf photosynthetic pigments, endogenous indole acetic acid (IAA), membrane stability index (MSI) in wheat plant as well as total carbohydrates of the yielded grains. Meanwhile, carotenoids, total phenolic content (TPC), total soluble sugars (TSS), proline, hydrogen peroxide (H2O2), lipid peroxidation product (MDA), membrane leakage (ML), and enzymatic antioxidant activities were increased compared with control plants. Moreover, the antioxidant enzymes activity and flavonoids contents of the grains were increased in plants grown under 60% water irrigation requirements (WIR). While, SNP and H2O2 treatments improved the reduced impact of water stress on wheat growth and yield through enhancing photosynthetic pigments, RWC, IAA, osmolytes accumulation and antioxidant enzymes activities. Moreover, treatments with SNP and H2O2 caused marked decreases in H2O2, MDA levels and membrane leakage percentages (ML%). Priming with SNP or H2O2 improved wheat water deficiency tolerance as evident by the increments in the growth and grain productivity of water stressed wheat plant.

Key words: Antioxidant enzymes, osmolytes, signal molecules, water deficiency, wheat, yield.

Introduction

Wheat (*Triticum aestivum* L.) is among the largest cereal crop all over the world and is one of the main sources of calories and protein. It has a unique role in people's diets, trade, economy and country politically [1]. Nevertheless, wheat production in Egypt increased in recent years, its production satisfies only 45% of its annual domestic demand, so Egypt still one of the main wheat importing countries. Consequently, wheat production must be increased to reduce the gap between the output and consumption [2]. On the other hand, wheat is often farmed in arid and semi- arid zones where a lack of adequate water can result in production losses of up to 29% [3]. Accordingly, exploring the respond of wheat plant to drought is of great significance issue especially with the accelerated climate changes which induced serious drought stress [4]. Water is essential for some stages of plant life including early plant growth, development, and grain formation [5].

Water deficiency induces a lot of physiological and biochemical responses in many crops, which let plant to adapt to such limiting environmental conditions [6]. It reduces plant photosynthesis, induces variations of chlorophyll contents and constituents [7]. It also decrease photochemical and enzymes activities of Calvin cycle [8]. Water-stress-promoted overproduction of reactive oxygen species (ROS). These ROS are the main cause of damages occurred to lipids, proteins, photosynthetic pigments, and nucleic acids of plant cells [9]. At cellular levels, the major organelles or sites of ROS production are chloroplasts, vacuoles, micro bodies, and mitochondria [10]. Plants which have a good – established can evolve an defense antioxidant mechanism (enzymatic and non-enzymatic) for detoxifying and neutralizing excess ROS [11 &12]. Enzymatic antioxidants assist in

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holding protection against oxidative stress. Superoxide dismutase (SOD) is the major antioxidant enzyme involved in the conversion of ROS into H_2O_2 which will be buffered by peroxidase (POD) and catalase (CAT) enzymes [10]. Regarding to non-enzymatic antioxidants, carotenoids, ascorbic acid (AsA) and phenolics are known as major significant antioxidant group because their antioxidant characters [13]. To mitigate the harmful effects of water defici1ency stress, plants have also evolved an osmotic adjustment process via increasing the biosynthesis of osmolytes as glycine betaine, proline, free amino acids, secondary metabolites, and total soluble carbohydrates [14]. Osmotic homeostasis exerts its effect through establishing plant development and cell turgor via lowering osmotic potential, causing increased growth [15].

Sensing of biotic or abiotic stress conditions causes signaling cascades that trigger ROS formation, calcium, nitric oxide, overproduction of growth regulators such as abscisic acid, ethylene, jasmonic acid, and salicylic acid. Consequently, those signals stimulate expression of certain subsets of defense genes which cause the assembly of all defense reaction [16]. Plants respond to stresses in a synergistic way as single cells and as a whole plant. Plant cell membrane sensors are the first to detect stress signals. The signal information is subsequently passed downstream, causing a number of stress-response genes to be activated. Pre-soaking of seeds with different signal molecules has been recorded earlier on increasing abiotic stress plant tolerance. Nitric oxide (NO) and hydrogen peroxide (H_2O_2) are of these signal molecules, they have amazing prospect of increasing stress tolerance [17], either by activation of antioxidant systems or by increase accumulation of osmolytes.

Sodium nitroprusside (SNP, nitric oxide source), controls a lot of plant physiological and developmental mechanisms [18], plant water relations [19], seed germination [20], photosynthesis, stomatal conductance [21], floral regulation, fruit ripening, and senescence [22]. Nitric oxide NO is an antioxidant that can reduce the oxidative injury [23 & 24].

Hydrogen peroxide (H_2O_2), one of ROS, is considered as a major cellular metabolite. H_2O_2 is continuously synthesized in plant via different sources involving enzyme and non- enzyme mechanisms. Recently, it has been recognized that H_2O_2 displayed significant effect in plant developmental and several physiological processes. Hydrogen peroxide (H_2O_2) is the most stable ROS, and have the ability to diffuse across the subcellular membrane very fast [25]. In plant cells H_2O_2 plays a dual role, at high levels, it disrupts photosynthesis and promotes programmed cell death [26], meanwhile at low level, it serves as a second messenger via collaborating with a variety of important signal molecules [27]. It controls gene expression in plant defence system [28], decrease lipid peroxidation [27], enhance membrane stability [29] and controls lot of physiological mechanisms [30]. Furthermore, H_2O_2 can serve as a secondary messenger in signal transduction processes [31].

So this investigation aimed to improve the antioxidant defence systems as well as the osmoregulatory strategy, which in turn mitigate water stress injuries and enhance wheat growth and productivity.

Experimental

Experiment was carried out in the greenhouse of National Research Centre, Dokki, Cairo, Egypt (29.77 N, 31.3 E), in winter season of 2018/2019 and 2019/2020. The experimental plant was wheat (*Triticum aestivum* L.) cultivar Giza 168. Wheat grains were obtained from Agricultural Research Centre, Giza, Egypt. The two signal molecules H_2O_2 and sodium nitroprusside (SNP) used were obtained from Sigma-Aldrich Co, USA. Wheat grains were soaked for 12 hours (the most suitable time) in various levels of sodium nitroprusside or H_2O_2 (0, 50 and 100 µM) and denoted as C, SNP1, SNP2, H_2O_2 1, H_2O_2. Soaked air dried grains were sown in November during two successive seasons, in central row in plastic pots each, filled with approximately 7 kg clay soil from. The soil was mixed with yellow sand in a ration 3:1 to reduce compaction and improve drainage (v/v). The pots were divided into two main groups based on the amount of water irrigation requirement, 100 percent and 60 percent (WIR). Each of the two groups were divided into five subgroups of different treatments of either SNP or H_2O_2 at concentrations 0, 50 and 100 µM (C, H_2O_2 1, H_2O_2 2, SNP1 and SNP2,). Each treatment composed of 4 replicates distributed in a Randomized Complete Block Design RCBD. Seedlings were watered twice a

week by two different water irrigation requirement 100% and 60% WIR. Thinning was done after 15 days and five plants were left in each pot. Fertilization were added at the recommended doses of super phosphate, potassium sulfate, and urea in each pot. Samples were randomly chosen from each treatment after 45 days from sowing to examine growth attributes and biochemical parameters. Three plants/pot were left for yield determination. Shoot length (cm), leaves numbers/plant, fresh and dry weight of shoot and root (g/plant) and relative water content were determined. At full maturity, yield and its attributes were measured (plant height (cm), biological yield (g/plant), spike length (cm), spike weight (g), spikelet number/spike, grains weight/plant (g), 1000 grains weight (g), carbohydrates (%), DPPH activity and flavonoids contents.

Water irrigation Requirement:

Two water irrigation requirements were calculated using Penman Monteith equation and crop coefficient according according to Allen [32]. The average amount of irrigation water applied calculated (100% and 60%) for two seasons after deducting the amount of rainwater that fell during the two growing seasons for wheat, (*Triticum aestivum* L.) variety Giza 168.

Relative water content (RWC):

Relative water contents was also measured and expressed as percentage according to the following equation [33]:

RWC (%) = (Fresh weight – Dry weight) / Fresh weight x 100

Biochemical analysis

Chlorophyll a, chlorophyll b and carotenoids were measured according to Lichtenthaler and Buschmann [33]. Relative water content (%) was determined according to Alqurainy [34]. Indole acetic acid (IAA) was determined as described by Larsen [35]. Hydrogen peroxide level was estimated according Yu [36]. Lipid peroxidation was evaluated by estimating malondialdehyde (MDA) content according to Hodges [37]. Membrane leakage (ML) was estimated according to Vahala [38]. Membrane stability index (MSI) was estimated by Sairam [39]. Enzymes were extracted using Chen and Wang [40] method. Peroxidase (POX) (EC 1.11.1.7) activity was determined as the method described by Kumar and Khan [41] method. Catalase (CAT) (EC 1.11.1.6) activity was determined according [40]. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute [42]. Superoxide dismutase (SOD) (EC 1.12.1.1) activity was determined by Chen and Wang [40] method. The activity of nitrate reductase (NR, EC 1. 7. 1. 1) was measured according to Jaworski [43]. The NR activity was expressed as nano moles of nitrite produced per gram fresh weight per hour (nM NO_2/g FW h^{-1}). Phenol content was extracted and analyzed as described by Danil and George [44]. Total soluble carbohydrates (TSS) were extracted as described by Homme [45] and determined by Albalasmeh [46]. Proline contents was extracted according to the method described by Vartainan [47] and assayed according to Bates [48]. Determination of total carbohydrates was extracted according to Herbert [49] and assayed according to Smith [50]. Free radical scavenging activity was done according to Gyamfi [51]. Flavonoid content of crude extract was determined as the method described by Chang [52].

Statistical analysis:

The analyzed data were represented by calculating the mean of four replicates with the standard deviation (± SD). The analysis of variance (ANOVA) of the data and the least significant difference test (LSD, $P \leq 0.05$) for the comparison of means were performed by SAS ® [53]. The Least significant differences (LSD) were calculated according to Steel [54].

Results

Growth indices:

The effect of pre-soaking wheat grains in different concentrations (0.0, 50 and 100 µM) of either SNP or H_2O_2 on growth indices of wheat plant grown under water deficiency is presented in Table (1). It is obvious that decreasing water irrigation requirement from 100% to 60% WIR (water deficiency significantly decreased growth indices (shoot length (cm), leaves number/plant, shoot and root fresh and dry weight (g)) as well as relative water content of wheat plant. The percentages of decreases in shoot length (cm), leaves number/plant, shoot and root fresh and dry weight reached about 14.21%, 19.76%, 10.38%, 13.64%, 16.83%, and 18.75% respectively. Regarding to SNP or H_2O_2 treatment it is clear that, SNP was superior in increasing shoot length, dry weight of root and relative water (RWC) over H_2O_2 treatments.

Meanwhile, H_2O_2 was superior in increasing leaves number/plant, shoot fresh and dry weight as well as root dry weight of wheat plant. Furthermore, Table (1) clearly shows that, different levels of SNP or H_2O_2 caused significant increases in the studied growth indices. These gradual increments in the investigated growth parameters were positively related to SNP or H_2O_2 concentrations (Table 1).

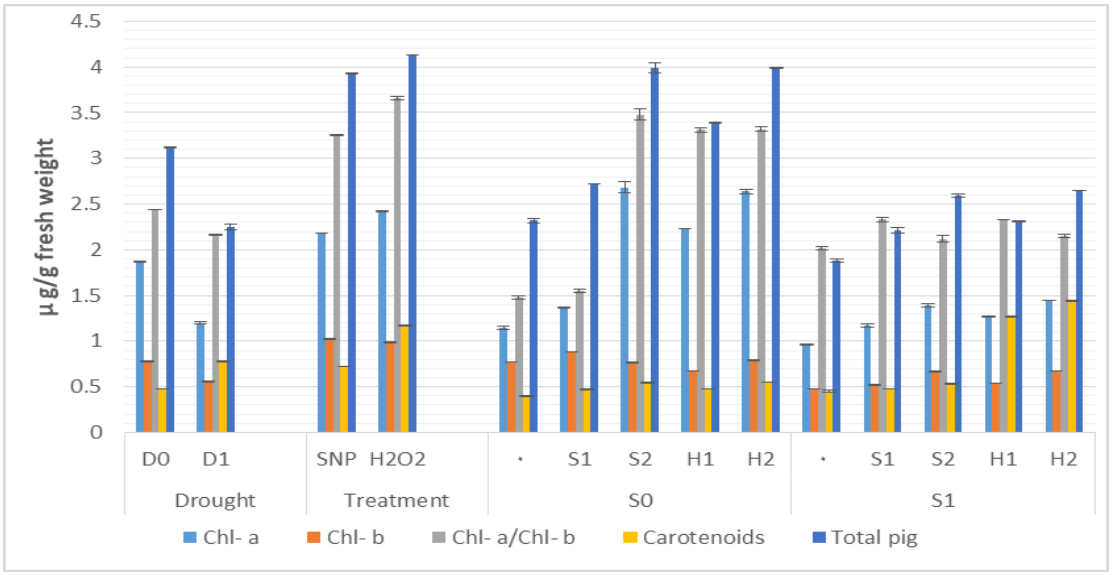
Photosynthetic pigments and endogenous indole acetic acid (IAA):

Water deficiency significantly decreased chlorophyll a (*Chl*- a), chlorophyll b (*Chl*- b), the ratio between *Chl*- a/*Chl*- b and total pigments (Fig 1) as well as endogenous indole acetic acid content (Fig 2). Meanwhile, carotenoids content of stressed wheat leaves were markedly increased (Fig 1). With respect to SNP or H_2O_2 treatments, it is clear that, SNP was superior in increasing *Chl*- b, while, H_2O_2 was superior in increasing *Chl*- a, *Chl*- a/ *Chl*- b carotenoids and total pigments of wheat leaf. Data presented in Fig. 1 and 2 clearly showed that, different levels of SNP or H_2O_2 caused significant increases in the components of photosynthetic pigments and endogenous IAA contents compared with their untreated control plants (0). Higher concentrations of SNP or H_2O_2 were more effective than lower concentration (Fig 1 & 2).

Table (1): Growth indices of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR. Data are means of four replicates during two seasons.

	Treatment	Shoot length (cm)	Leaves no/tiller	Shoot fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry wt (g)	RWC%
	D0	42.6±0.434	5.0±0.129	1.83±0.009	0.44±0.003	0.410±0.009	0.112±0.002	76.96±1.425
	D1	36.6±0.249	4.0±0.047	1.64±0.010	0.38±0.002	0.341±0.008	0.091±0.001	75.25±1.625
	LSD at 5%	1.244	0.64	0.09	0.03	0.033	0.001	0.734
	SNP	80.3±0.425	6.75±0.059	2.54±0.022	0.62±0.006	0.562±0.007	0.152±0.002	76.25±1.025
	H_2O_2	78.0±0.328	6.92±0.156	2.66±0.015	0.63±0.008	0.582±0.006	0.149±0.002	75.20±0.698
	LSD at 5%	2.150	0.14	0.08	0.02	0.021	0.004	0.2145±
	0	38.7±1.247	4.7±0.236	1.61±0.017	0.40±0.011	0.317±0.011	0.094±0.002	75.16±2.236
	SNP1	43.0±0.409	5.0±0.000	1.77±0.036	0.42±0.013	0.405±0.010	0.105±0.005	76.27±2.356
	SNP2	48.3±1.247	5.3±0.236	2.13±0.017	0.50±0.009	0.501±0.022	0.133±0.002	76.53±2.542
	H_2O_2 1	43.3±0.624	5.3±0.236	1.72±0.046	0.42±0.009	0.413±0.008	0.113±0.005	75.58±0.986
D0	H_2O_2 2	43.7±0.471	5.3±0.236	2.14±0.078	0.51±0.001	0.509±0.006	0.131±0.003	76.17±1.365
	0	32.7±0.236	3.7±0.236	1.27±0.039	0.32±0.011	0.272±0.002	0.081±0.003	74.80±1.524
	SNP1	37.3±0.471	4.0±0.000	1.63±0.089	0.40±0.031	0.322±0.016	0.093±0.001	75.46±0.985
	SNP2	41.0±0.408	4.33±0.236	1.77±0.041	0.43±0.011	0.432±0.006	0.101±0.000	75.71±1.625
	H_2O_2 1	36.3±0.913	4.00±0.000	1.77±0.0256	0.40±0.03	0.322±0.016	0.093±0.002	75.46±2.315
D1	H_2O_2 2	39.3±1.247	4.67±0.236	1.79±0.040	0.43±0.010	0.426±0.001	0.096±0.001	75.98±1.854
	LSD at 5%	1.807	0.59	0.15	0.09	0.116	0.006	0.405

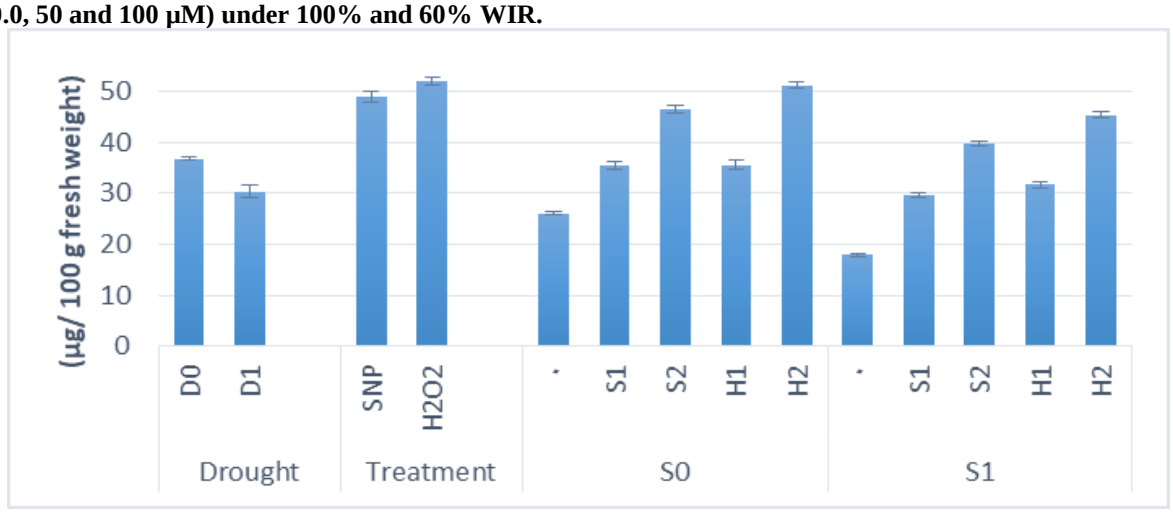
(Each value represents the mean ± standard error (n=4)



(Each value represents the mean ± standard error (n=4).

LSD at 5% *Chlo* a (Drought: 0.006, SNP and H_2O_2: 0.03, Interaction: 0.062), *Chlo* b (Drought: 0.004, SNP and H_2O_2: 0.004, Interaction: 0.012), *Chlo* a/*Chlo* b (Drought: 0.03, SNP and H_2O_2: 0.04, Interaction: 0.06), carotenoids (Drought: 0.01, SNP and H_2O_2: 0.01, Interaction: 0.02) and total pigments (Drought: 0.08, SNP and H_2O_2: 0.03, Interaction: 0.06).

Figure (1): Photosynthetic pigments (µ g/g fresh weight) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR.



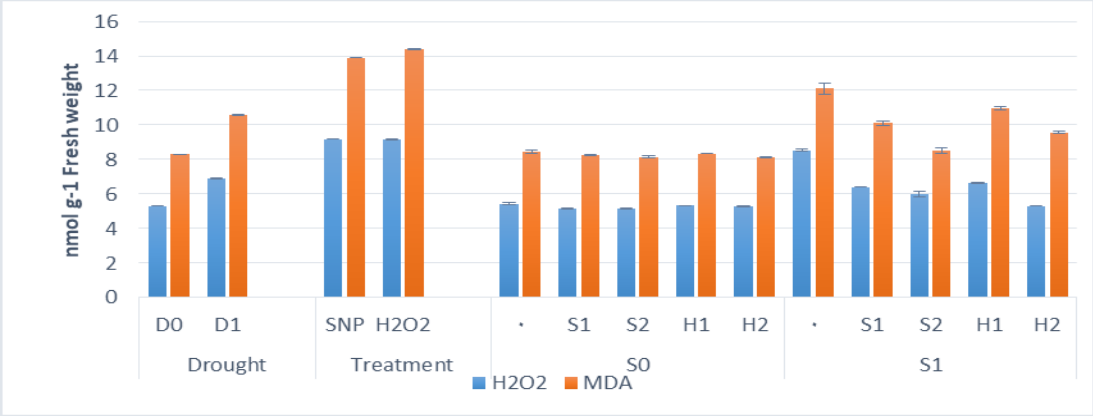
(Each value represents the mean ± standard error (n=4).

LSD at 5% (Drought: 0.70, SNP and H_2O_2: 1.21 and interaction: 2.72)

Figure (2): Endogenous IAA (µg/100 g fresh weight) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) grown under 100% and 60% WIR.

Hydrogen peroxide (H_2O_2) and MDA contents, membrane leakage (ML), membrane stability index (MSI):

Imposition of water deficiency significantly ($p \leq 0.005$) enhanced accumulation of hydrogen peroxide (H_2O_2) and malonaldahyde (MDA) in wheat leaves (Fig 3). H_2O_2 priming stimulated the accumulation of leaf H_2O_2 and MDA in wheat leaves as compared to SNP treatment. SNP and H_2O_2 at different levels significantly ($p \leq 0.005$) reduced H_2O_2 and MDA accumulation in wheat leaves either under normal or water stress conditions. Among different treatments 100 µM of SNP was the most effective followed by 100 µM H_2O_2 as compared with other treatments.



(Each value represents the mean ± standard error (n=4).
LSD at 5% H_2O_2 (Drought: 0.187, SNP and H_2O_2: 0.037, Interaction: 0.144), MDA (Drought: 0.434, SNP and H_2O_2: 0.06, Interaction: 0.39).

Figure (3): H_2O_2 (nmol g^{-1} FW) and MDA (nmol (MDA) g^{-1} FW) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR.

Water deficincy also significantly ($p \leq 0.005$) increased membrane leakage (ML) (Fig 4a) in wheat leaf, meanwhile significantly decreased membrane stability index (MSI) (Fig 4b) compared with non-stressed plants. Grain priming in SNP caused more significant decreases in leaf ML and MSI compared with H_2O_2treatment. Moreover, priming of wheat grains with different concentrations of either SNP or H_2O_2 caused significant decline ($p \leq 0.005$) in ML under normal or water deficit conditions (Fig 4a).

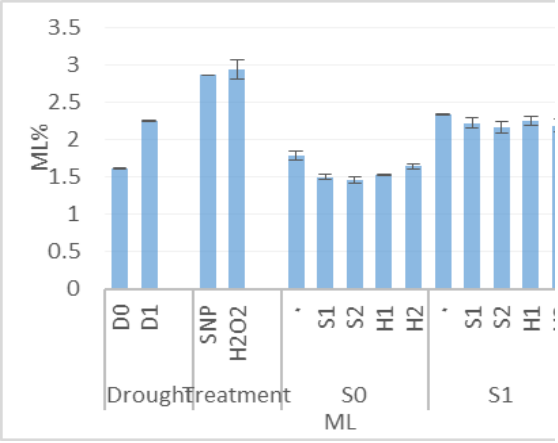


Figure 4a

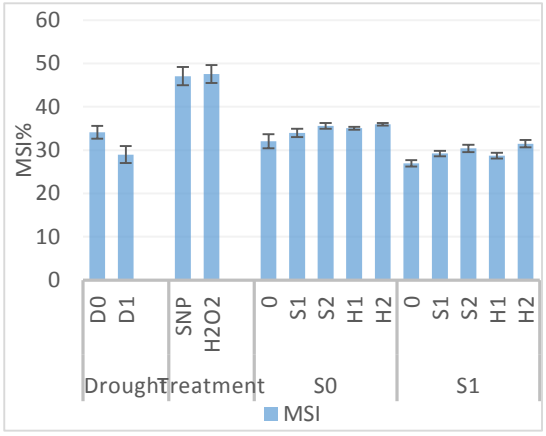


Figure 4b

(Each value represents the mean ± standard error (n=4).
LSD at 5%: ML% (Drought: 0.05, SNP and H_2O_2: 0.01, Interaction: 0.06), MSI% (Drought: 0.05, SNP and H_2O_2: 0.01, Interaction: 0.57).

Figure (4a and b): Membrane leakage (ML%), membrane stability index (MSI%) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR.

Antioxidant enzymes:

Exposing wheat plants to water deficincy (60% WIR) significantly ($p \leq 0.005$) enhanced the activities of some antioxidant enzymes such as peroxidase POX, catalase CAT, super oxide dismutase SOD and nitrate reductase NR of wheat plant (Table 2). Priming wheat grains in different concentrations of SNP and H_2O_2 significantly ($p \leq 0.005$) increased the above mentioned enzymes activities in wheat plant either under normal (100% WIR) or water deficit conditions (60% WIR). It is clear from Table (2) that increasing concentrations of either SNP or H_2O_2 caused more increases in the studied enzymes activities as compared with their corresponding untreated controls.

Among different treatments 100 µM of SNP was the most effective followed by 100 µM H_2O_2 compared with other treatments. Priming wheat grains in SNP gave more activities of antioxidant enzymes of wheat plant than H_2O_2 treatment.

Total phenolic content TPC, total soluble sugars (TSS) and proline contents:

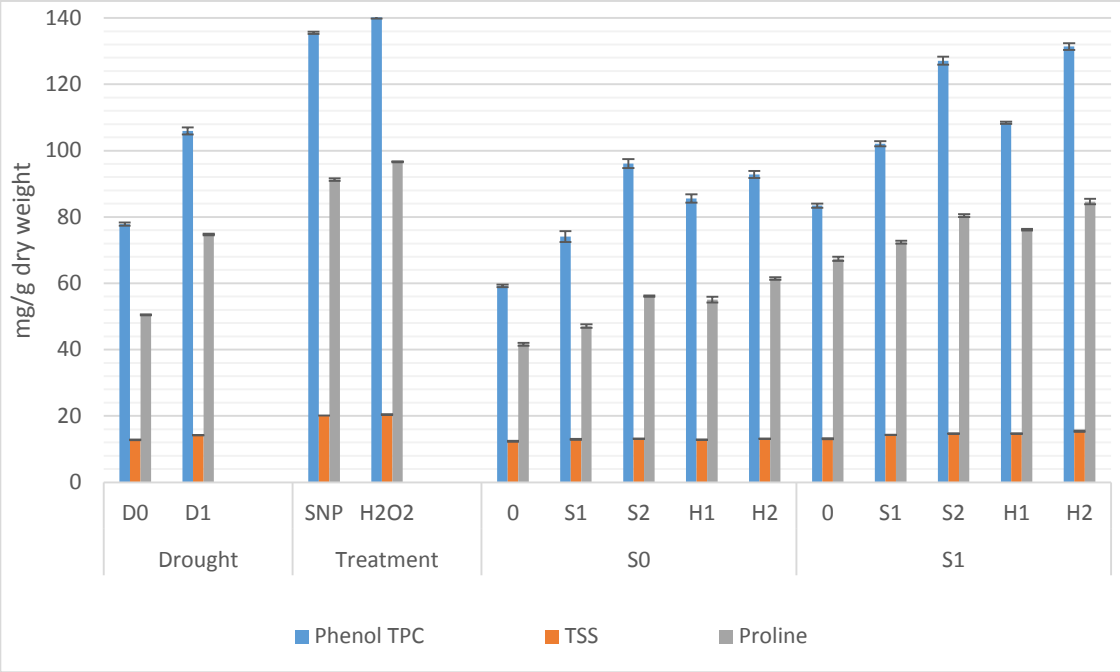
Water deficincy significantly increased total phenolic contents, TPC and some osmolytes as TSS and proline contents of wheat plant. Data in Fig (5) also showed that there are significant differences between SNP or H_2O_2 treatments in phenolic, TSS and proline contents of wheat plants. Priming wheat grains with H_2O_2 was superior as compared to SNP treatment in the accumulation of the above mentioned parameters.

Regarding to grain priming in SNP or H_2O_2 with different concentrations (50 & 100 µM) under normal irrigation water (100% WIR) and water stress (60% WIR), SNP or H_2O_2 treatments caused significant further accumulation of TPC, TSS and proline contents in water stressed wheat plants. A similar improvement in TPC, TSS and proline contents was also recorded in unstressed wheat plants treated with SNP or H_2O_2 (Fig 5). Higher concentrations of both SNP and H_2O_2 treatment were more effective in comparison with lower concentrations under both water-stressed and non-stressed conditions (Fig 5).

Table (2): Antioxidant enzymes POX, CAT, SOD (U/min/g Fresh wt.) and NR (nM NO_2 / g FW h^{-1}) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR.

Treatment		POX	CAT	SOD	NR
D0		19.31±0.059	66.36±0.173	33.10±1.487	259±1.487
D1		36.52±0.596	85.65±0.451	56.00±1.194	275±1.194
LSD at 5%		0.30	1.96	1.16	3.56
SNP		42.76±0.113	115.59±0.383	68.53±1.177	404±1.766
H_2O_2		40.99±0.043	112.42±0.267	65.11±0.378	399±0.378
LSD at 5%		0.26	0.80	0.66	2.46
D0	0	15.72±0.047	56.27±0.985	23.32±0.149	236.36±0.406
	SNP1	18.68±0.00	65.25±0.653	34.25±0.245	261.90±0.592
	SNP2	24.61±0.392	80.68±0.408	46.10±0.102	291.35±2.041
	$H_2O_2$1	18.10±0.102	61.45±0.369	30.06±0.196	251.15±1.021
	H_2O_2 2	23.07±0.239	78.25±0.572	41.55±0.327	282.50±0.469
D1	0	26.56±0.088	67.00±0.674	38.15±0.082	260.85±1.023
	SNP1	36.32±0.259	94.10±0.306	61.22±0.639	274.50±0.347
	SNP2	49.17±0.198	99.08±0.163	71.09±0.994	293.40±1.245
	H_2O_2 1	34.27±0.135	90.50±0.363	58.30±0.149	279.07±2.348
	$H_2O_2$2	46.25±0.163	96.22±0.218	69.07±0.169	286.35±0.0
LSD at 5%		0.52	1.63	0.97	4.76

(Each value represents the mean ± standard error (n=4).



(Each value represents the mean ± standard error (n=4)
LSD at 5%: Phenol (Drought: 2.80, SNP and H_2O_2: 0.78, Interaction: 2.89), TSS (Drought: 0.16, SNP and H_2O_2: 0.108, Interaction: 0.405), proline (Drought: 1.05, SNP and H_2O_2: 0.49, interaction: 1.19).

Figure (5): Total phenolic contents TPC, TSS and proline contents (mg/100 g dry weight) of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR.

Yield components and quality:

The impacts of pre-soaking wheat grains in either SNP or H_2O_2 with different concentrations (0.0, 50 and 100 µM) on yield components and quality including plant height (cm), biological yield/plant (g), spike length (cm), spikelet number/spike, grains weight/plant (g), 1000 grains weight (g) were presented in Table (3). In addition, the nutritional value of the yielded grains as carbohydrates%, DPPH% and flavonoids contents were presented in Table (4) of wheat plant grown under water deficit. Data clearly showed that, decreasing water irrigation requirement from 100% to 60% WIR (water deficiency) caused significant decreases in various yield indices as compared with those plants irrigated normally (100% WIR). Table (3 and 4) showed that, pre-soaking wheat grains in H_2O_2 was more effective than SNP. Furthermore, data presented in Table (3 & 4) showed that different concentrations of either SNP or H_2O_2 not only caused promotive effect on different yield components and the nutritional values of the yielded grains in plants irrigated with 100% WIR, but also, alleviated the reduced effect of water deficit compared with untreated controls. Higher levels of either hydrogen peroxide or sodium nitropurrside were more effective than lower ones.

Table (3): Yield and its indices of wheat plants primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR. Data are means of four replicates of two seasons

Treatment		Plant height (cm)	Biological yield (g)	Spike length (cm)	Grains weight/plant (g)	Spikelet no/spike	Spike weight (g)	1000 Grains weight
D0		62.539±0.758	1.49±0.011	10.667±0.334	1.336±0.036	9.833±0.501	2.332±0.0584	31.91±0.549
D1		55.000±0.040	1.36±0.003	9.056±0.134	1.148±0.011	9.056±0.166	2.039±0.0367	27.40±0.728
LSD at 5%		1.606	0.05	0.370	0.017	0.874	0.049	2.25
SNP		86.942±0.291	2.12±0.004	14.417±0.047	1.853±0.011	13.667±0.057	3.280±0.031	43.99±0.340
H2O2		89.367±0.256	2.15±0.003	15.667±0.118	1.863±0.006	15.083±0.156	3.287±0.027	45.36±0.153
LSD at 5%		0.827	0.02	0.423	0.030	0.481	0.016	0.49
	0	58.00±0.817	1.430±0.011	9.667±0.236	1.233±0.031	8.333±0.236	2.263±0.035	28.760±0.544
	SNP1	62.83±0.425	1.483±0.018	10.333±0.236	1.317±0.016	9.000±0.00	2.356±0.044	31.657±0.716
	SNP2	66.27±0.961	1.520±0.012	11.333±0.471	1.420±0.039	10.667±0.236	2.407±0.022	33.483±0.515
	H_2O_2 1	62.97±0.796	1.493±0.005	10.67±0.234	1.367±0.010	10.333±0.236	2.303±0.035	33.160±0.185
D0	H_2O_2 2	67.17±0.425	1.567±0.023	12.333±0.259	1.443±0.026	12.333±0.234	2.397±0.033	35.660±0.339
	0	48.67±0.236	1.237±0.0.016	7.667±0.237	1.040±0.029	7.667±0.237	1.867±0.185	23.940±0.935
	SNP1	55.67±0.965	1.390±0.016	9.000±0.00	1.163±0.033	9.000±0.408	2.033±0.014	27.227±0.213
	SNP2	56.33±0.236	1.420±0.007	9.667±0..00	1.237±0.009	10.000±0.00	2.193±0.024	30.880±0.446
	H_2O_2 1	60.33±0.849	1.390±0.012	9.000±0.408	1.163±0.012	9.000±0.234	2.033±0.004	27.227±0.155
D1	$H_2O_2$2	60.33±1.247	1.467±0.005	11.333±0.471	1.247±0.048	11.000±0.408	2.240±0.020	31.207±0.272
LSD at 5%		2.101	0.036	3.224	0.031	2.886±	0.225±	4.718

(Each value represents the mean ± standard error (n=4).

Table (4): Nutritional values (carbohydrates%, DPPH% and flavonoids contents mg/100 g dry wt) of wheat grain yield primed with SNP and H_2O_2 (0.0, 50 and 100 µM) under 100% and 60% WIR. Data are means of four replicates of two seasons

Treatment		CHO %	DPPH %	Flavonoids
D0		45.99±0.129	56.44±0.700	67.49±0.519
D1		44.98±0.035	71.05±0.256	78.42±0.299
LSD at 5%		0.19	0.87	1.00
SNP		68.00±0.068	92.35±0.109	107.66±0.819
H_2O_2		68.45±0.019	98.89±0.013	111.20±0.470
LSD at 5%		0.10	0.24	0.96
	0	45.30±0.049	46.60±0.350	51.87±0.382
	SNP1	45.62±0.039	56.96±0.431	65.52±0.414
	SNP2	46.53±0.043	64.22±1.678	76.21±1.081
	H_2O_2 1	46.31±0.174	58.21±0.226	72.38±0.287
D0	$H_2O_2$2	46.89±0.063	66.06±0.106	87.09±0.732
	0	44.39±0.050	57.07±0.331	63.73±0.955
	SNP1	44.87±0.089	67.32±0.189	81.12±0.414
	SNP2	45.30±0.089	77.22±0.458	92.20±1.369
	H_2O_2 1	45.48±0.0272	81.70±0.242	84.16±1.369
D1	$H_2O_2$2	45.43±0.074	85.93±0.134	85.56±0.191
LSD at 5%		0.25	0.71	1.97±

(Each value represents the mean ± standard error (n=4)

Discussion

In the current study, decreasing WIR to 60% significantly reduced wheat growth and yield. Water deficit is regarded to decrease wheat growth and productivity due to decreased photosynthetic pigment [55]. As a result of the disruption in this mechanism, the assimilating pathway is disrupted, thus lowered seed production [56]. Similar work has been done by Sadak and Bakry [57], and Sadak [58], Abd Elhamid [59] they stated that, water deficit-induced decreases in flax, wheat and moringa growth and productivity. While, grain soaking in SNP and H_2O_2 enhanced growth and yield of wheat plant under water deficit. Gao [60] and Orabi and Sadak [61] confirmed the positive role of H_2O_2 treatment on growth of canola and wheat plants respectively under stress. Furthermore, Ramadan [62] reported that exogenous treatment with sodium nitroprusside SNP enhanced sunflower growth and productivity. These increases in response to SNP may be due to its role in increasing photosynthetic rate under oxidative stress [63] as reflected by the increases in chlorophyll contents. Ashraf [64] found a similar relation between photosynthesis with maize growth under drought by H_2O_2 treatment. In our investigation, leaf relative water contents were reduced in response to decreasing WIR to 60% in wheat plant. However SNP and H_2O_2 primed plants showed significant increases in RWC under normal (100% WIR) and water stressed (60% WIR). Habib and Ashraf [19] stated that, SNP application enhanced RWC in water stressed rice plants. The improvement of RWC by H_2O_2 treatment was recorded in water stressed soybean [65]. Those improvement in cellular water relations could be attributed to quick accumulation of compatible solutes that could effectively nullify the cellular osmotic stress [66].

Significant reductions in leaf photosynthetic pigments contents of wheat plant grown under water deficit were noticed in the current investigation. Similar results were obtained by Ashraf [64] on maize, Orabi & Sadak [61] on wheat, Sun [55] on cucumber and Ramadan [62] on sunflower. The improved roles of NO and H_2O_2 in chlorophyll biosynthesis could be attributed to its impact on inducing chlorophyll biosynthesis and / or decrease breakdown, also due to protection of chloroplast membranes through protecting chloroplastic membrane, also their impact on promoting plant antioxidative defense system [60] by acting as signal molecules.

H_2O_2 level was stimulated in wheat plant exposed to water deficit. Similar results have been reached by Ali [67]. Meanwhile, SNP and H_2O_2 pretreatments significantly decreased the accumulation of H_2O_2 in wheat plants subjected to water deficit. Zhang [23] and Ramadan [62] stated that NO treatment could reduce accumulation of H_2O_2 of *Populus euphratica* and sunflower plants grown under water stress. Similarly, Orabi and Sadak [61] stated that exogenous treatment with H_2O_2 decreased the accumulation of H_2O_2. Furthermore, cellular membrane is among the most prevalent cell targets under various stress states [68]. The extent of membrane injury represents the plant´s ability to tolerate stresses like drought. Water stress stimulated significant lipid peroxidation and ML accompanied by MSI reduction. The present data are in accordance with those of Fazeli [69] on water stressed sesame. Also, Jamil [70] found that water deficit significantly decreased MSI in different wheat cultivars. In addition, it has been observed that electrolyte leakage of leaves of poplar plants was increased under drought conditions [71]. There is evidence suggesting that the principal sites of damage to cells and organelles are membranes [72 & 73]. Malondialdehyde (MAD) content, one of the degradation products of polyunsaturated fatty acids (PUFA) of bio membranes [74], was used to evaluate lipid peroxidation. PUFA are the principal membrane lipid constituents sensitive to peroxidation and degradation [75]. So, the increased MDA content may result via stomatal closure causing reduction in leaf CO_2 content. This, leading to a drop in the content of $NADP^+$ available to take electrons from PSI and/or PSII therefore initiate O_2 decreases with the concomitant production of ROS. Electrolyte leakage during water stress is thought to be caused by injury of cell membranes that become more porous as a result of lack of water [76]. Valentovic [77] confirmed the increments in electrolyte electrolyte leakage in water deficit stressed maize plant. Stress might decrease cell membrane stability enhancing membrane leakage level [78]. Lower membrane stability, concomitant with increased leakage, under drought might be attributed to stress effect on membranes protein denaturation [79]. Meanwhile, treatment with either SNP, H_2O_2 decreased MDA and H_2O_2, ML meanwhile increased MSI in wheat plant grown under drought stress. Zhang [23], and Habib [66] stated that treatment with SNP can reduce H_2O_2 and lipid peroxidation under drought. Moreover, NO

itself reacts with ROS as a chain terminator to suppress lipid peroxidation [80]. Farooq [81] showed, H_2O_2 treatment decreased MDA and H_2O_2 of wheat. Finally, those obtained decreases in response to H_2O_2 or SNP which confirmed their effective role as signal molecules [82].

Additionally, the activities of PPO, SOD and NR, were enhanced in wheat plant exposed to water stress. These improvements were previously reported by Sadak and Bakry [57] in stressed flax plant. These enhanced enzyme activities were displayed by SNP and H_2O_2 treatments under normal and drought-stressed conditions, confirming their role on controlling ROS accumulation for keeping membrane's integrity, via stimulating activities of key antioxidant enzymes under water-stressed conditions [83]. The results of Habib [66] are in agreement with our results. Nitric oxide (NO) and H_2O_2 as effective ROS scavengers could directly react with oxygen species and oxidize/reduce O^-_2/O_2•- to O_2/ H_2O_2 via enhancing oxygen utilization in mitochondrial respiratory chain [84]. It was also reported that NO and H_2O_2 may indirectly promote antioxidant system through up-regulating the expression of antioxidant enzymes which leads to enhanced activities of these enzymes, thereby improving plant tolerance against drought stress [85]. More recently, alleviation of oxidative stress via improving the activities of antioxidant enzymes such as SOD, CAT, and APX have been reported by application of NO and H_2O_2 [58].

Water deficit stress increased the accumulation of total phenolic contents. Exogenous treatment of SNP and H_2O_2 were helpful in improving phenolic contents in water-stressed and normal irrigated wheat plants. Phenolics considered as powerful antioxidant scavenger of free radicals via high reactivity as electron or hydrogen donors [86]. The improved phenolics contents in response to water deficit might resulted from the disruption in many metabolic pathways [87]. The efficient effect of NO in the induction of antioxidant system might explain the enhanced phenolics increases caused by SNP [88]. Habib and Ashraf [19] using SNP and Orabi & Sadak [61] using H_2O_2 confirmed our results.

Total soluble sugars considered as ROS scavengers thus improving membrane stabilization. Moreover, TSS stabilizes water content, osmotic adjustment to maintain turgor. Proline is one of osmoprotectants, secondary metabolite and considered as an antioxidant [89]. During osmotic stress, increased proline level works as water replacement stabilizing cell components via their hydrophobic interactions and hydrogen bonding, preventing membrane dehydration [66]. In the present study, a similar increasing trend in cellular TSS and proline contents were observed in SNP or H_2O_2- pretreated wheat plant. Those osmoprotectants accumulation by NO are in agreements with Dong [24], and Ramadan [62]. Alia [90] stated that proline acts as a powerful $^1O_2$ quencher.

Decreasing water irrigation requirement from 100% to 60% decreased significantly total carbohydrates of the yielded wheat grains, meanwhile increased antioxidant activities and flavonoids contents. The decreases in total carbohydrates content are associated with reductions in growth as presented in Table 1, as well as the photosynthetic pigments content as mentioned in Fig 1. Moreover, these decreases in carbohydrates are very crucial as due to their direct relationship with different physiological processes as photosynthesis, translocation of nutrients and respiration [66]. Meanwhile, the investigated pretreatments improved carbohydrates content as well as antioxidant activities and flavonoids contents of the yielded grains, these increases might be resulted from increased growth parameters, photosynthetic pigments as mentioned in Table 1 and Fig 1. Furthermore, these increases in carbohydrates contents reflected to the increased output of photosynthesis in leaves therefore, increased their translocation from leaves to the developing grains. Flavonoids are regarded as plant secondary metabolites having an antioxidant activity. The antioxidant activity depends on the presence of free OH groups, especially 3-OH. The improvement of flavonoids in response to drought stress can reflect some kind of defense strategy against stress conditions.

Conclusions

The reduced impact caused by water stress could be alleviated by SNP and H_2O_2 grain soaking in wheat. Sodium nitroprusside and H_2O_2 improve wheat plants tolerance to drought via improving the antioxidant system. They generally affected ROS scavenging activity and increased the enzymatic antioxidants level. Furthermore, exogenous treatments improved vegetative growth, grain yield in wheat, increased photosynthetic pigments, endogenous IAA and other secondary metabolites content, additionally, the decrease in the hydrogen peroxide and malondialdehyde level in wheat leaves confirmed the

protective roles of SNP and H_2O_2 against cell membrane damage under water stress conditions.

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