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Variable Responses of Seedlings of Two Wheat Cultivars towards Exogenous Hydrogen Peroxide

Manpreet Kaur[1] and Vikramjit Kaur Zhawar[1*]

[1]Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, 141004, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author MK designed the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author VKZ managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Recent reports have shown that hydrogen peroxide (H_2O_2) is an important signaling molecule and that interacts with abscisic acid (ABA) signalling for some of its physiological effects.
Study Design: This work was conducted to study H_2O_2 regulation of seed germination, growth and antioxidant potential in two wheat cultivars varying in drought tolerance and ABA-sensitivity. Sodium tungstate as ABA biosynthetic inhibitor was used to evaluate the effects of H_2O_2 mediated through ABA biosynthesis.
Place and Duration of Study: Laboratory of Department of Biochemistry, Punjab Agricultural University, Ludhiana, 141004 India, between June 2012 and September 2012.
Methodology: Two wheat cultivars C306 (ABA higher sensitive and drought tolerant) and PBW343 (ABA lesser sensitive and drought susceptible) were used. 50 mM H_2O_2 with and without sodium tungstate (an inhibitor for ABA biosynthesis) was supplied exogenously for 24-48 h to 4-day old seedlings. Data was taken at 24 and 48 h after treatment.
Results: Exogenous H_2O_2 delayed germination in both cultivars. It increased shoot and root

**Corresponding author: E-mail: vikram97jit@pau.edu;*

biomass in C306 but not in PBW343. It increased antioxidant potential in terms of increased activities of antioxidant enzymes and proline contents in both cultivars but such increases were significantly higher in C306. It also led to production of some level of oxidative stress in terms of increased contents of H_2O_2, malondialdehyde (MDA), and decreased levels of ascorbate contents and ascorbate/dehydroascorbate ratio in both cultivars but level of such oxidative stress was higher in PBW343. These H_2O_2-regulated effects were affected by inhibiting ABA biosynthesis in both cultivars; however, the effects were higher in C306 as compared to PBW343.

Conclusion: H_2O_2-regulations were higher in wheat cultivar C 306 than PBW 343. Such regulations were partly mediated through ABA biosynthesis.

Keywords: Abscisic acid; antioxidant; hydrogen peroxide; Triticum aestivum.

1. INTRODUCTION

Reactive oxygen species (ROS) are recently known for their signaling functions. Among them, H_2O_2 being non-toxic and having longer half-life, may act as signal for a wide variety of of plant functions. H_2O_2 signaling activates antioxidant potential in plant [1], acts downstream to ABA-signaling [2,3,4,5], and functional under abiotic stresses like water stress and salt stress [1,5]. H_2O_2 signaling and ABA-signaling interacts with each other during seed germination [6] and stomatal closure [7].

In our previous work [8], wheat cultivars PBW343 and C306 were compared for antioxidant response under water stress with or without ABA. This included antioxidant enzymes, ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), guaiacol peroxidase (GPOX), levels of ascorbate, dehydroascorbate, H_2O_2, MDA and proline. PBW343 showed decreases of H_2O_2 and poor upregulation of antioxidant potential under water stress but showed improvement when ABA was supplied exogenously. C306 showed increases of H_2O_2, followed by upregulation of antioxidant potential under water stress. In many studies [9,10,11, 12], H_2O_2 supply or H_2O_2-pretreatment have been found to alleviate stress (heat, salt and drought) effects by increasing antioxidant potential and decreasing oxidative toxicity. This study was to measure the antioxidant responses in these cultivars under exogenous H_2O_2. These cultivars were found to vary in ABA-sensitivity and showed differential responses towards various environmental stresses, salt stress, water stress, heat stress, exogenous sugar supply [8,13,14,15,16,17].

Studies [1,2,3,4,5] have shown ABA produces H_2O_2 through activation of NADPH oxidase, and then H_2O_2 activates antioxidant potential through a MAPK-pathway. Other reports have shown that H_2O_2 can also induce ABA production through increasing ABA biosynthesis as found in root tips of wheat seedlings [18] and in germinating barley embryos [19]. In this study, sodium tungstate was used under H_2O_2 as an inhibitor of ABA biosynthesis to check if H_2O_2-regulation is affected on inhibiting ABA biosynthesis. Sodium tungstate is widely used as inhibitor of ABA biosynthesis [17,20]. It inhibits abscisic aldehyde oxidase, an enzyme in the ABA biosynthetic pathway.

2. MATERIALS AND METHODS

Seeds were germinated in 50 mM H_2O_2, 5 mM sodium tungstate in 50 mM H_2O_2 (H_2O_2+ST) and autoclaved distilled water (CT). G. I. (Germination index) which depicts rate of germination, was calculated using equation,

$$G.\ I. = \sum_{i=1}^{k} ni/ti \ ,$$

where ni was % of seeds germinating on ith day and ti was number of days counted from start of the experiment, where k was the last day of the experiment.

Seeds of wheat (*Triticum aestivum* L.) cultivars PBW343 and C306 were disinfected with 0.1% $HgCl_2$, sown on filter paper moistened with autoclaved distilled water in sterilized petri dishes and kept at 25ºC for 4 days. Treatment was applied on the 4th day as 50 mM H_2O_2, 5 mM sodium tungstate in 50 mM H_2O_2 (H_2O_2+ST) and water as control (CT). Shoot and root fresh biomasses of 25 seedlings were measured at 48 h after treatment. Antioxidant enzymes were measured at 24 and 48h post treatment as in Kaur et al. 2014 [8]. In brief, common extraction was made in 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 2% PVP and 0.05% triton-X-100 and used for all assays. APX was assayed in 50 mM potassium phosphate buffer of pH 7.0, 0.1 mM EDTA, 0.3 mM ascorbate, 1 mM H_2O_2 at 290 nm for disappearance of ascorbate ($_{ascorbate}$ = 2.8 mM-1 cm-1). CAT was assayed in 50 mM

potassium phosphate buffer pH 7.0 and 25 mM H_2O_2, at 240 nm for disappearance of H_2O_2 ($_{H2O2}$ = 0.0394 mM-1 cm-1). GPOX was assayed in 100 mM potassium phosphate buffer pH 6.5, 50 mM guaiacol and 32 mM H_2O_2 at 470 nm for appearance of tetraguaiacol ($_{tetraguaiacol}$ = 26.6 mM-1 cm-1). GR was assayed in 50 mM potassium phosphate buffer (pH 7.0), 0.7 mM GSSG, 0.07 mM NADPH at 320 nm for disappearance of NADPH($_{NADPH}$ = 6.22 mM-1 cm-1). All enzymes were assayed at 25°C. Antioxidants and other related metabolites were measured [8] at 24 and 48 h post treatment. In brief, H_2O_2 was extracted in 0.1% TCA, incubated in 1.3 M potassium iodide, 33 mM potassium phosphate buffer pH 7 at room temperature for 1 hr in the dark and read at 390 nm. Reduced ascorbate was extracted in 5% TCA, incubated in 0.8% H_3PO_4, 0.04% $FeCl_3$, 0.2% bipyridyl, 80% ethanol at 37°C for 40 min and read at 525 nm. Dehydroascorbate (oxidised ascorbate) was extracted in 5% metaphosphoric acid, 1% thiourea, incubated at 37°C in 1% dinitrophenyl hydrazine, 0.2% thiourea, 0.025% $CuSO_4•5H_2O$, 4.5N H_2SO_4 for 3 h, further incubated at room temperature in 61% H_2SO_4 (cold) and read at 530 nm. Proline was extracted in 3% sulphosalicylic acid, incubated in 48 mM ninhydrin, 9.3 M acetic acid, 0.8 M phosphoric acid at 100°C for 1 h, phase-separated at room temperature by adding equal volume of toluene, upper layer of pink-red color read at 520 nm. MDA was extracted in 0.1% TCA, incubated at 100°C in 0.4% thiobarbituric acid, 16% TCA for 30 min, read at 532 nm and 600 nm where absorbance at 600 nm was subtracted from absorbance at 532 nm and calculated using molar extinction coefficient of 155 mM-1 cm-1.

For each measurement, three biological replicates were taken. Mean ± SD was calculated. Data was analyzed by Duncan Multiple test at P 0.05 for statistical differences.

3. RESULTS

Exogenous H_2O_2 delayed seed germination (G. I.) in both cultivars (Table 1). This action of H_2O_2 did not involve ABA biosynthesis as germination was not found to have improved under H_2O_2+ST.

Exogenous H_2O_2 increased shoot and root growth in C306 but did not alter growth in PBW343. Under H_2O_2+ST, root growth was affected in C306 and shoot growth was affected in PBW343 (Table 1).

In PBW 343, exogenous H_2O_2 increased only APX in roots at 24h otherwise this supply did not increase any of the four antioxidant enzymes studied (Table 2). H_2O_2-increase of APX in roots of PBW343 at 24h was decreased under H_2O_2+ST. In C306, H_2O_2 application increased activities of many antioxidant enzymes; CAT, GR and SOD increased in roots at 48h, APX increased in roots at 24h, APX and GPOX increased in shoots at both 24 and 48h and GR increased in shoots at 24h. H_2O_2-induction of these antioxidant enzymes was decreased under H_2O_2+ST. We observed decrease of GPOX in shoots at 24 and 48h, APX in shoots at 48h and CAT in roots at 48h under H_2O_2+ST. It was clear that H_2O_2-induction of antioxidant enzymes was higher in C306 and this induction involved ABA biosynthesis.

Exogenous H_2O_2 decreased the redox state of ascorbate i.e. it decreased ascorbate and increased dehydroascorbate, thus decreasing the ascorbate to dehydroascorbate ratio in both cultivars. However, the decrease of redox state of ascorbate was much higher in PBW343 than C306 (Table 3).

Table 1. Effect of exogenous H_2O_2 and effect of inhibiting ABA biosynthesis under H_2O_2 on seed germination and growth of wheat cultivars. 50 mM of H_2O_2, 5 mM sodium tungstate (ABA biosynthetic inhibitor) in 50 mM H_2O_2 (H_2O_2+ST) were given to 4-day old seedlings where CT was water control. Data was collected at 48 h after treatment

Cultivar	Treatment	Germination index	Shoot FW (g)	Root FW (g)
PBW343	CT	156.8±0.50^{a}	1.19±0.12^{bc}	0.62±0.01^{bc}
	H_2O_2	148.9±1.56^{b}	1.29±0.10^{b}	0.51±0.05^{c}
	H_2O_2+ST	149.5±1.37^{b}	0.92±0.09^{cd}	0.52±0.02^{c}
C306	CT	124.5±5.27^{c}	0.83±0.03^{d}	0.59±0.06^{bc}
	H_2O_2	109.1±5.26^{d}	1.61±0.33^{a}	0.87±0.11^{a}
	H_2O_2+ST	74.6±0.53^{e}	1.35±0.05^{ab}	0.66±0.06^{b}

†Germination index (n=3) depicts rate of germination as discussed in Methods section
‡Shoot and root fresh weight (FW) in g per 25 plants (n= 3)
§Different letters represent significant difference according to Duncan Multiple test (0.05)

Table 2. Effect of exogenous H_2O_2 and effect of inhibiting ABA biosynthesis under H_2O_2 on ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), guaiacol peroxidase (GPOX) activities in shoot and root of wheat cultivars PBW343 and C306 at 24h and 48h of treatment given to 4d-old seedlings. Treatments were 50 mM H_2O_2, 5 mM sodium tungstate in 50 mM H_2O_2 (H_2O_2+ST) where ST is inhibitor of ABA biosynthesis. CT is water control

		PBW343			C306		
Shoot							
Enzyme	**Stage**	**CT**	**H_2O_2**	**H_2O_2+ST**	**CT**	**H_2O_2**	**H_2O_2+ST**
APX	24h	$0.81±0.03^{bcd}$	$0.37±0.01^{f}$	$0.62±0.05^{e}$	$0.73±0.06^{d}$	$0.85±0.06^{bc}$	$0.79±0.02^{cd}$
	48h	$0.85±0.04^{bc}$	$0.86±0.05^{bc}$	$0.80±0.03^{cd}$	$0.87±0.03^{bc}$	$1.18±0.09^{a}$	$0.92±0.13^{b}$
CAT	24h	$20.2±5.42^{bc}$	$18.7±0.32^{cd}$	$25.0±7.56^{ab}$	$11.2±1.75^{e}$	$12.9±3.16^{de}$	$12.0±1.05^{e}$
	48h	$19.5±3.1^{bc}$	$15.6±0.77^{cde}$	$18.2±0.85^{cd}$	$26.3±1.01^{a}$	$16.1±1.04^{cde}$	$15.9±2.02^{cde}$
GR	24h	$18.0±5.78^{cd}$	$18.0±6.64^{cd}$	$22.0±2.85^{bc}$	$24.2±4.61^{bc}$	$35.1±5.33^{a}$	$39.1±6.54^{a}$
	48h	$26.9±1.5^{b}$	$21.1±1.39^{bc}$	$22.1±6.54^{bc}$	$22.1±2.42^{bc}$	$21.9±1.39^{bc}$	$11.6±0.51^{d}$
GPOX	24h	$2.03±0.47^{de}$	$1.96±0.23^{de}$	$1.87±0.53^{def}$	$1.54±0.16^{efg}$	$3.01±0.18^{ab}$	$2.09±0.15^{d}$
	48h	$1.46±0.12^{fgh}$	$1.11±0.11^{gh}$	$1.00±0.33^{h}$	$2.29±0.10^{cd}$	$3.27±0.23^{a}$	$2.66±0.11^{bc}$
Root							
APX	24h	$1.87±0.06^{d}$	$4.80±0.38^{a}$	$2.83±0.47^{c}$	$1.90±0.31^{d}$	$3.23±0.17^{b}$	$3.57±0.13^{b}$
	48h	$1.55±0.01^{de}$	$1.04±0.10^{fg}$	$1.23±0.08^{ef}$	$1.06±0.08^{fg}$	$0.80±0.02^{g}$	$0.74±0.14^{g}$
CAT	24h	$5.44±0.57^{d}$	$5.19±0.37^{d}$	$5.32±0.22^{d}$	$21.2±1.12^{c}$	$21.0±3.05^{c}$	$20.4±2.87^{c}$
	48h	$9.47±0.49^{d}$	$5.75±0.78^{d}$	$7.32±0.46^{d}$	$22.5±2.42^{c}$	$57.3±5.80^{a}$	$33.3±3.96^{b}$
GR	24h	$31.2±10.5^{bc}$	$30.7±3.67^{bc}$	$35.7±9.81^{ab}$	$42.9±2.48^{a}$	$26.6±1.63^{bc}$	$32.0±1.51^{b}$
	48h	$27.9±2.6^{bc}$	$11.4±2.88^{d}$	$11.8±3.26^{d}$	$22.0±2.41^{c}$	$36.1±5.72^{ab}$	$44.6±5.82^{a}$
GPOX	24h	$11.3±1.87^{a}$	$11.1±0.21^{a}$	$8.29±0.91^{b}$	$5.72±0.20^{de}$	$5.20±0.05^{de}$	$6.25±0.06^{cd}$
	48h	$8.80±0.72^{b}$	$7.36±0.19^{bc}$	$4.14±1.63^{e}$	$8.32±0.12^{b}$	$12.5±0.51^{a}$	$9.00±1.08^{b}$

†Values are Mean ± S. D. of three replicates and represent activities of APX as μ mole of ascorbate changed min^{-1} mg^{-1} protein, CAT as μ mole of H_2O_2 changed min^{-1} mg^{-1} protein, GR as η mole of NADPH changed min^{-1} mg^{-1} protein and GPOX as μ mole of tetraguaiacol changed min^{-1} mg^{-1} protein.

‡ Different letters in each double lined set represent significant difference according to Duncan Multiple test (0.05)

Table 3. Effect of exogenous H_2O_2 supply and effect of inhibiting ABA biosynthesis under H_2O_2 on reduced and oxidised ascorbate contents in shoot and root of wheat cultivars PBW343 and C306 at 24h and 48h of treatment given to 4d-old seedlings. Treatments were 50 mM H_2O_2, 5 mM sodium tungstate in 50 mM H_2O_2 (H_2O_2+ST) where ST is inhibitor of ABA biosynthesis. CT is water control

		PBW343			C306		
Shoot							
	Stage	**CT**	**H_2O_2**	**H_2O_2+ST**	**CT**	**H_2O_2**	**H_2O_2+ST**
ascorbate	24h	0.626 ± 0.05^{g}	0.352 ± 0.02^{h}	0.355 ± 0.03^{h}	1.39 ± 0.08^{e}	0.898 ± 0.02^{f}	1.25 ± 0.08^{e}
	48h	2.72 ± 0.24^{a}	1.92 ± 0.11^{d}	1.03 ± 0.03^{f}	2.30 ± 0.065^{b}	2.15 ± 0.063^{c}	1.98 ± 0.019^{d}
dehydroascorbate	24h	$1.68 \pm .073^{g}$	3.06 ± 0.259^{e}	3.50 ± 0.525^{d}	0.302 ± 0.033^{i}	0.280 ± 0.008^{i}	0.299 ± 0.021^{i}
	48h	1.24 ± 0.069^{h}	2.47 ± 0.121^{f}	2.88 ± 0.305^{e}	4.28 ± 0.224^{c}	4.67 ± 0.110^{b}	5.61 ± 0.190^{a}
ascorbate /dehydroascorbate ratio	24h	0.372	0.115	0.101	4.62	3.20	4.20
	48h	2.19	0.780	0.356	0.537	0.460	0.353
Root							
ascorbate	24h	0.516 ± 0.02^{e}	0.065 ± 0.007^{i}	0.080 ± 0.007^{i}	0.402 ± 0.024^{f}	0.267 ± 0.008^{g}	0.190 ± 0.011^{h}
	48h	1.30 ± 0.121^{a}	0.636 ± 0.038^{d}	1.12 ± 0.063^{b}	0.962 ± 0.014^{c}	0.444 ± 0.006^{ef}	0.478 ± 0.012^{ef}
dehydroascorbate	24h	0.989 ± 0.087^{d}	0.366 ± 0.022^{h}	0.650 ± 0.024^{fg}	1.38 ± 0.10^{b}	0.565 ± 0.016^{g}	0.612 ± 0.013^{fg}
	48h	0.799 ± 0.094^{e}	0.559 ± 0.050^{g}	0.738 ± 0.091^{ef}	1.88 ± 0.094^{a}	1.16 ± 0.108^{c}	1.18 ± 0.039^{c}
ascorbate /dehydroascorbate ratio	24h	0.521	0.178	0.123	0.291	0.473	0.311
	48h	1.63	1.14	1.51	0.512	0.383	0.403

† Values are Mean ± S. D. of three replicates and represent contents of ascorbate or dehydroascorbate as mg g^{-1} DW.
‡ Different letters in each double lined set represent significant difference according to Duncan Multiple test (0.05)

Exogenous H_2O_2 increased H_2O_2 content (Table 4) in shoots and roots of both cultivars. Increases of H_2O_2 were higher in shoots of PBW343 than of C306. On inhibiting ABA biosynthesis, H_2O_2 was increased in shoots and roots of C306 at 24 and 48h but in PBW343, H_2O_2 was increased only in roots at 48h.

Exogenous H_2O_2 increased MDA only in shoots of PBW 343, otherwise it did not increase MDA in roots of both cultivars. Under H_2O_2+ST, MDA was increased in roots of both cultivars (24h in PBW343 and 48h in C306).

Exogenous H_2O_2 increased proline in shoots (24 and 48h) and roots (48h) of C306 while in PBW343, such increase was observed only in shoots at 48h. On inhibiting ABA biosynthesis, proline was decreased in shoots and roots of C306 but not in PBW343.

4. DISCUSSION

Exogenous H_2O_2 is found to activate germination in seeds of *Arabidopsis thaliana* [6]. We found delay of germination by H_2O_2 in both cultivars. This delay could be due to as H_2O_2 is involved in cell wall crosslinking reactions. This was found in seeds of *Lepidium sativum* [21] where 10 mM of exogenous H_2O_2 inhibited the weakening of the endosperm cap and produced atypical germination in approximately 10% of seeds. Moreover, different concentrations of H_2O_2 and the stage of its application have also affected the response. For example, pre-treatment of pea seeds with 5-100 mM H_2O_2 for 12-24h before imbibition (in water) increased germination while germination of same seeds in 10-20 mM H_2O_2 was reduced [22,23].

We found H_2O_2 increase of growth in C306 similar to reported finding in pea seedlings [22] where H_2O_2-pretreatment of pea seeds increased growth of seedlings in concentration dependent manner. Growth was decreased by inhibition of ABA biosynthesis under H_2O_2. This indicated that H_2O_2-upregulation of growth may involve ABA biosynthesis.

Our finding that H_2O_2 increased activity of antioxidant enzymes was similar to other reports where exogenous H_2O_2/ H_2O_2-pretreatment was found to increase activity as well as gene expression like SOD activity and MnSOD, Cu/Zn SOD genes expression in leaves of wheat. seedlings [24]; increased APX, GR activities and

Table 4. Effect of exogenous H_2O_2 and effect of inhibiting ABA biosynthesis under H_2O_2 on H_2O_2, malondialdehyde (MDA) and proline contents in shoot and root of wheat cultivars PBW343 and C306 at 24h and 48h of treatment given to 4d-old seedlings. Treatments were 50 mM H_2O_2, 5 mM sodium tungstate in 50 mM H_2O_2 (H_2O_2+ST) where ST is inhibitor of ABA biosynthesis. CT is water control

		PBW343			C306		
Shoot							
	Stage	**CT**	**H_2O_2**	**H_2O_2+ST**	**CT**	**H_2O_2**	**H_2O_2+ST**
H_2O_2	24h	$2.78±0.07^{e}$	$3.83±0.11^{c}$	$1.87±0.14^{g}$	$1.43±0.05^{h}$	$1.95±0.12^{fg}$	$2.21±0.02^{f}$
	48h	$6.38±0.39^{b}$	$11.8±0.30^{a}$	$6.35±0.12^{b}$	$2.21±0.07^{f}$	$3.34±0.05^{d}$	$4.01±0.10^{c}$
MDA	24h	$27.1±1.94^{d}$	$46.1±3.76^{c}$	$44.3±2.81^{c}$	$6.60±0.25^{f}$	$10.1±0.58^{ef}$	$8.60±0.10^{f}$
	48h	$28.7±3.58^{d}$	$80.0±12.6^{a}$	$56.9±2.46^{b}$	$12.4±0.56^{ef}$	$16.4±0.96^{e}$	$13.4±0.32^{ef}$
proline	24h	$7.42±0.44^{f}$	$5.74±0.36^{f}$	$7.78±0.32^{f}$	$12.4±1.86^{de}$	$27.1±0.81^{b}$	$18.1±0.46^{c}$
	48h	$7.46±0.456^{f}$	$11.8±0.145^{e}$	$11.5±0.05^{e}$	$14.7±1.51^{d}$	$35.2±4.49^{a}$	$28.9±0.74^{b}$
Root							
H_2O_2	24h	$2.70±0.19^{ef}$	$2.26±0.14^{fg}$	$2.61±0.09^{ef}$	$1.03±0.10^{h}$	$1.94±0.12^{g}$	$3.13±0.04^{e}$
	48h	$6.09±0.14^{d}$	$11.4±0.81^{b}$	$13.2±0.56^{a}$	$3.08±0.19^{e}$	$6.35±0.18^{d}$	$9.82±0.58^{c}$
MDA	24h	$32.2±2.68^{b}$	$11.4±1.98^{e}$	$18.2±3.28^{d}$	$32.7±1.56^{ab}$	$35.8±1.50^{a}$	$31.1±0.62^{b}$
	48h	$6.09±0.29^{f}$	$5.87±0.53^{f}$	$5.61±0.39^{f}$	$26.3±2.14^{c}$	$21.4±3.02^{d}$	$29.5±2.36^{bc}$
proline	24h	$9.06±0.44^{g}$	$4.53±0.02^{h}$	$6.33±0.19^{h}$	$19.6±0.72^{d}$	$19.3±0.81^{d}$	$13.1±0.57^{e}$
	48h	$10.1±0.11^{fg}$	$9.45±0.07^{g}$	$11.9±0.62^{ef}$	$23.8±3.27^{c}$	$31.6±0.65^{a}$	$27.7±2.96^{b}$

† Values are Mean ± S. D. of three replicates and represent contents of H_2O_2 and proline as μ mole g^{-1} DW, MDA as ηmole g^{-1} DW

‡ Different letters in each double lined set represent significant difference according to Duncan Multiple test (0.05)

expression of their genes in roots of rice seedlings [25]; and increased APX, POX and ascorbate oxidase in pea seedlings [22]. Under stresses, activation of antioxidant enzymes involved H_2O_2 as mediator. This was observed in *Ulva fasciata* [26] for increase of FeSOD under salt stress and in maize roots [27] for increases of APX under spermidine supply. Pre-treatment of seeds or plants with H_2O_2 before exposing to heat and salt stress in rice [9], salt stress in maize [10] and wheat [11] or H_2O_2 spraying before drought in soybean [12] have led to improved tolerance due to increased antioxidant activity and decreased toxicity. H_2O_2 thus serves as a signalling molecule to activate antioxidant enzymes.

Increase of proline was found under H_2O_2 in this study. According to another report [28], ABA increase of proline may be mediated through H_2O_2. Decrease of reduced ascorbate and reduced ascorbate to dehydroascorbate ratios and increase of MDA under H_2O_2 application indicated the production of oxidative stress. Decrease of the redox state of ascorbate was reported in H_2O_2-pretreated pea seedlings using 5-20 mM concentration of H_2O_2 [23]. Increase of MDA was also reported in wheat leaf under 0.05-0.2mM H_2O_2 [29].

Higher antioxidant potential (antioxidant enzymes and proline) accompanied with lesser oxidative stress (in terms of increased H_2O_2, MDA and decreased redox state of ascorbate) were observed in C306 than PBW343. This indicates that the antioxidant mechanism of C306 has a higher level of H_2O_2-upregulation. Previously, drought susceptibility of PBW343 was related to absence of increases of H_2O_2 followed by lesser antioxidant potential under water stress [8], but this study showed that even if H_2O_2 is supplied, induction of antioxidant activity in PBW343 is not to the same level as in C306. This shows that H_2O_2-regulation is somehow influenced by the ABA sensitivity of the cultivar. Moreover, a decrease of antioxidant potential and increase of oxidative stress were observed on inhibiting ABA biosynthesis under H_2O_2 in both cultivars and such changes were higher in C306. This supports the view that ABA biosynthesis plays role in H_2O_2-upregulation of antioxidant activity.

5. CONCLUSION

In summary, this study shows that exogenous H_2O_2 may slow down seed germination in seeds of wheat. Exogenous H_2O_2 increases antioxidant enzymes, proline, H_2O_2, MDA and decreases redox state of ascorbate in shoots and roots of wheat seedlings. C306 shows higher levels of antioxidant activity and lesser oxidative stress under H_2O_2 as compared to PBW343. H_2O_2-regulation of antioxidant response is influenced by ABA-biosynthesis/ABA-pathway and shows its lesser level in ABA-lesser sensitive cultivar than ABA-higher sensitive cultivar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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