DOI: 10.1007/s10535-013-0312-6 BIOLOGIA PLANTARUM 57 (3): 487-494, 2013

Effect of hydrogen peroxide on seedling growth and antioxidants in two wheat cultivars

J. LU[1,2], X.N. LI[1], Y.L. YANG[1]*, L.Y. JIA[1], J. YOU[1], and W.R. WANG[1]

School of Life Science, Northwest Normal University, Lanzhou 730070, P.R. China[1]
Ningbo College of Health Sciences, Ningbo 315100, P.R. China[2]

Abstract

This study aimed to investigate seed germination, seedling growth, and antioxidative responses in two wheat cultivars, Ningchun and Xihan, exposed to different H_2O_2 concentrations. Ningchun exhibited higher germination rate but lower root and shoot growth than Xihan when exposed to H_2O_2 treatment. Assays using fluorescent dye H_2DCFDA and propidium iodide showed a significantly enhanced H_2O_2 content and a cell elongation inhibition in H_2O_2-treated roots. The malondialdehyde content was elevated with increasing exogenous H_2O_2 concentration. Moreover, treatments of seedlings with H_2O_2 scavenger, catalase (CAT), and antioxidant, butylated hydroxytoluene, partly abolished H_2O_2-induced negative effect on root growth. In both untreated and H_2O_2-treated leaves, SOD activity in Ningchun was higher than that in Xihan, but POD and APX activities in Ningchun were lower than those in Xihan leading to elevated H_2O_2 level in Ningchun leaves but decreased H_2O_2 content in Xihan ones under H_2O_2 treatment.

Additional key words: ascorbate peroxidase, catalase, glutathione reductase, *Triticum aestivum*, superoxide dismutase.

Introduction

Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) can rapidly inactivate enzymes, damage cellular organelles, and destroy membranes, proteins, lipids, and nucleic acids which may result in plant cell death (Karuppanapandian *et al.* 2011). On the other hand, H_2O_2 acts as a secondary messenger that controls various plant processes such as growth and development, stress responses, and programmed cell death (Gechev *et al.* 2006, Li *et al.* 2009). Although Foyer *et al.* (1994) reported that the direct H_2O_2 application to growing plants was lethal due to its strong oxidizing property, the protective role of exogenous H_2O_2 against abiotic stresses was observed (He *et al.* 2009, Gondim *et al.* 2010). H_2O_2 was able to promote germination (Christophe *et al.* 2008) or formation and development of adventitious roots (Li *et al.* 2009). However, Lin and Kao (2001) reported that exogenous H_2O_2 from 2.5 to 10 mM resulted in the increase of endogenous H_2O_2 content and inhibited root growth in rice seedlings.

The response of plants to stress conditions was accompanied by increased activities of antioxidant enzymes (Candan and Tarhan 2012) including superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), glutathione reductase (GR, EC 1.6.4.2), and ascorbate peroxidase (APX; EC 1.11.1.11). Among the enzymes, SOD is a metalloprotein that catalyzes the dismutation of superoxide anion to H_2O_2 and molecular oxygen in the cytosol, mitochondria, and chloroplasts (Fridovich 1986). CAT, POD, GR, and APX represent main elimination H_2O_2 systems in plant cells (Mittler 2002). Recently, several studies showed the stimulation of antioxidant systems in wheat seedlings (Li *et al.* 2011), red kidney bean roots (Liu *et al.* 2012), and *Nitraria tangutorum* callus (Yang *et al.* 2012) when exposed to exogenous H_2O_2. However, the negative effects of H_2O_2 on antioxidant enzyme activities were observed in other plants (Peng *et al.* 2008, Ozden *et al.* 2009).

Received 30 March 2012, *accepted* 14 November 2012.
Abbreviations: APX - ascorbate peroxidase; ASA - ascorbate; BHT - butylated hydroxytoluene, CAT - catalase, EDTA - ethylenediaminetetraacetic acid; GR - glutathione reductase; H_2DCFDA - 2',7'-dichlorodihydrofluorescein diacetate; MDA - malondialdehyde; NBT - nitroblue tetrazolium; PI - propidium iodide; POD - peroxidase; SOD - superoxide dismutase.
Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (No. 31160088) and Program for New Century Excellent Talents at University of China.
* Author for correspondence; fax: (+86) 931 7971575, e-mail: xbsfyangyingli@163.com

Wheat is one of the most important agricultural crops in many countries. Xihan 2 (Xihan) and Ningchun 4 (Ningchun) are different in drought tolerance. The aim of this study was to investigate seed germination, seedling growth, and antioxidative responses in these two wheat cultivars exposed to exogenous H_2O_2 stress. The present study was also undertaken to evaluate the mechanism of H_2O_2 negative effects on seedling growth.

Materials and methods

Two wheat (*Tritium aestivum* L.) cultivars Ningchun 4 and Xihan 2 purchased from Gansu Agricultural University and Gansu Agricultural Academy, respectively, were used. Ningchun 2 is drought-sensitive and Xihan 4 is drought-tolerant. The seeds were surface-sterilized with 0.1 % (m/v) $HgCl_2$ for 10 min, soaked in water for 24 h and then planted in Petri dishes with two filter-paper discs containing different H_2O_2 concentrations (0, 25, 50, 100, and 200 μM) and germinated at 25 ± 1.5 °C in the darkness in an incubator (*LRH-250-A*, *Medical Instruments Factory*, Guangdong, China) for 4 d. For root and shoot growth experiments, seedlings were cultivated in Petri dishes (9 cm in diameter) containing 6 cm^3 of 0 - 200 μM H_2O_2 solute (renewed every 2 d) for 6 d at 25 ± 2.5 °C and a 12-h photoperiod with irradiance of 300 μmol m^{-2} s^{-1}. For inhibition of H_2O_2 effects, H_2O_2 scavenger, catalase (CAT; 300 U dm^{-3}), and antioxidant, butylated hydroxytoluene (BHT; 200 μM), were used in wheat seedlings exposed to 50 and 200 μM H_2O_2. All assays were replicated at least three times to minimize experimental errors; each replicate was carried out on 50 seeds for germination and 40 seedlings for growth measurements.

The H_2O_2 in seedling roots was visualized using a fluorescent dye, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), as described by Pei *et al.* (2000). Roots were immersed in 50 μM H_2DCFDA in 10 mM Tris-HCl buffer (pH 7.2) containing 50 mM KCl at 37 °C for 20 min, washed three times in Tris-HCl buffer to remove excess dye, and then H_2DCFDA fluorescence was examined using a *Leica* (Germany) *DMIRB* inverted fluorescent microscope. Further, the roots were immersed in 10 μg cm^{-3} fluorescent dye propidium iodide (PI) in 10 mM phosphate buffer (pH 7.4) for 10 min, washed three times in phosphate buffer to remove excess dye, and examined by a laser scanning confocal microscope (*LSCM, LSM510 Meta*, *Zeiss*, Germany; excitation 488 nm, emission 525 nm).

The H_2O_2 content was measured as described by Sergiev *et al.* (1997). Plant leaves were ground in an ice bath with 2 cm^3 of 0.1 % (m/v) trichloroacetic acid. The homogenate was centrifuged at 12 000 *g* for 20 min and 0.7 cm^3 of the supernatant was mixed with 0.7 cm^3 of NaH_2PO_4/Na_2HPO_4 buffer (PBS; 10 mM, pH 7.0) and 0.7 cm^3 of 1 M KI. H_2O_2 content was estimated by measuring the absorbance at 390 nm based on a standard curve.

For lipid peroxidation analysis, malondialdehyde (MDA) content was measured according to the method of Zhou (2001) with some modifications. Seedling roots or leaves (0.5 g) were immediately homogenized in 5 cm^3 of 0.25 % (m/v) thiobarbituric acid, then heated at 98 °C for 30 min, quickly cooled on ice and then centrifugated at 10 000 *g* for 10 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm.

For assaying antioxidant enymes, 1 g of plant material was ground with 1 cm^3 of chilled PBS buffer (50 mM, pH 7.8) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 1 % (m/v) polyvinylpyrrolidone. After centrifugation at 15 000 *g* for 30 min, the supernatant was collected for the measurement of antioxidant enzyme activities.

SOD activity was estimated on the basis of the method described by Dhindsa and Matowe (1981). The homogenate was added to 3 cm^3 of a reaction mixture consisting of 50 mM PBS buffer (pH 7.6), 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), and 0.1 mM EDTA-Na_2. The reaction was started by the addition of 2 μM lactochrome. After irradiation at 25 °C for 30 min, the absorbance was recorded at 560 nm using *Agilent 8453* UV/visible spectrophotometer (USA). One unit (U) of activity was defined as the quantity of SOD required to produce 50 % inhibition of NBT reduction.

A modification of the method of Aebi (1974) was used to assay CAT activity. In brief, the homogenate was added to 3 cm^3 of 50 mM PBS buffer (pH 7.0). After 5 min pre-incubation at 25 °C, 15 mM H_2O_2 was added to start the reaction and the absorbance changes were recorded at 240 nm for 3 min. An absorbance change of 0.01 per min was defined as one unit of CAT activity.

POD activity was measured following a modification of the method of Rao *et al.* (1996). The homogenate was mixed with 3 cm^3 of reaction mixture containing 50 mM PBS buffer (pH 6.5) and 20 mM guaiacol. After pre-incubation at 25 °C for 5 min, 0.05 % (v/v) H_2O_2 was added to initiate the reaction. The absorbance changes at 470 nm within 3 min were recorded, and one unit of POD activity was defined as an absorbance change of 0.1 per min.

GR activity was measured by monitoring the oxidation of NADPH according to Donahue *et al.* (1997). The homogenate was mixed with 50 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, 0.5 mM oxidized glutathione and 0.15 mM NADPH). The absorbance changes at 340 nm within 3 min were recorded. One unit of GR activity was defined as an absorbance change of 0.1 per min.

For measuring APX activity, wheat leaves (1 g) were ground with 1 cm^3 of chilled 50 mM PBS buffer (pH 7.0) containing 1 mM EDTA and 1 mM ascorbate (ASA).

After centrifugation at 15 000 *g* for 30 min, the supernatant was collected for the measurement of APX activity according to Nakano and Asada (1981) with some modifications. The assay was carried out in a reaction mixture consisting of 50 mM PBS (pH 7.0), 0.5 mM ASA, 0.1 mM H_2O_2, and the homogenate. The absorbance changes at 290 nm were recorded at 25 °C for 1 min after the addition of H_2O_2. One unit of APX activity was defined as an absorbance change of 0.1 per min.

The amount of soluble proteins was estimated according to the method of Bradford (1976) using bovine serum albumin as a standard.

All values were represented by an average of at least three replicate measurements ± SE and the significance of differences between the control and the treatments was evaluated by Student's *t*-test.

Results

Higher H_2O_2 concentration (from 50 µM to 200 µM) led to a significant increase in the germination rate of Ningchun seeds whereas no difference of seed germination was found between 25 µM H_2O_2-treated seeds and the control (Table 1). Compared with the control, a slight but insignificant elevation was observed in the germination rate of Xihan seeds under different H_2O_2 concentrations. In addition, both Ningchun and Xihan seedlings exhibited significant decrease in the length of roots and shoots under H_2O_2 treatment (Table 1). Especially, Ningchun seedlings were more sensitive to exogenous H_2O_2. For example, 50 µM H_2O_2-treated seedlings showed about 73 and 32 % decrease in the root and shoot lengths in Ningchun but only 66 and 17 % reduction in Xihan, respectively.

A faint fluorescent signal was detected in untreated root demonstrating basic content of H_2O_2 in the root cells (Fig. 1*A*, *D*). When Ningchun seedlings were treated with

Table 1. The changes of seed germination and seedling growth in two wheat cultivars after treatment with different H_2O_2 concentrations. Means ± SE from at least five independent measurements. * and ** - significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively.

H_2O_2 [µM]	Ningchun			Xihan		
	germination [%]	root length [cm]	shoot length [cm]	germination [%]	root length [cm]	shoot length [cm]
0	87.33±3.05	4.89±0.55	8.23±0.57	86.00±4.00	5.18±0.71	9.31±0.45
25	87.33±9.45	1.52±0.40*	6.26±0.57*	86.00±2.00	3.30±0.10*	8.78±0.35
50	98.00±3.46*	1.33±0.39*	5.58±0.31*	89.00±1.00	1.76±0.35**	7.72±0.45
100	97.33±1.15*	0.38±0.09**	4.41±0.51*	93.00±3.00	1.21±0.26**	6.49±0.66*
200	96.67±4.16*	0.37±0.15**	4.23±0.29*	90.00±2.00	0.93±0.03**	5.38±0.32*

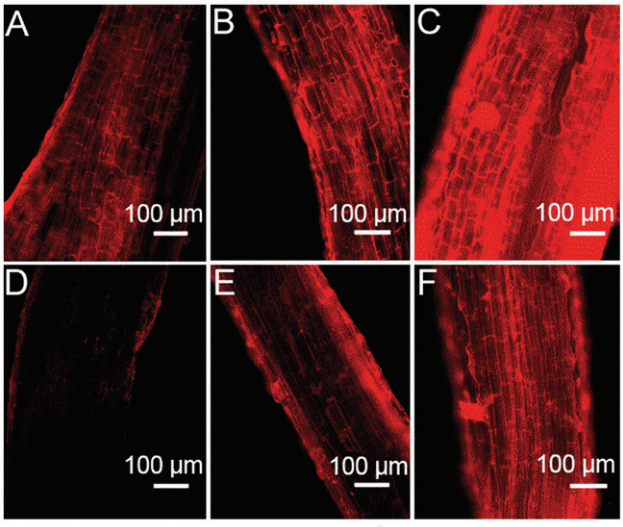

Fig. 1. Analysis of H_2O_2 content using a fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate in Ningchun (*A*, *B,* and *C*) and Xihan (*D*, *E,* and *F*) roots after treatment with different H_2O_2 concentrations for 6 d using *Leica DMIRB* inverted fluorescent microscope. *A* and *D* - control plants, *B* and *E* - 50 µM H_2O_2-treated plants, *C* and *F* - 200 µM H_2O_2-treated plants.

Table 2. The changes of endogenous H_2O_2 [ng g^{-1}(f.m.)] and MDA [μmol g^{-1}(f.m.)] content in roots and leaves of two wheat cultivars after treatment with different H_2O_2 concentrations for 6 d. Means ± SE from at least three independent measurements. * and ** - significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively.

H_2O_2 [μM]	Ningchun			Xihan		
	MDA (root)	H_2O_2 (leaf)	MDA (leaf)	MDA (root)	H_2O_2 (leaf)	MDA (leaf)
0	6.35±0.19	182.44±2.25	21.02±1.94	6.67±0.28	171.94±4.68	16.31±1.63
25	7.79±0.11*	205.06±1.37*	16.47±0.35	6.80±0.26	108.46±3.33**	19.54±0.89
50	7.75±0.99*	211.04±3.12*	20.80±4.81	7.83±0.50*	140.78±4.88*	15.64±1.06
100	11.03±0.14**	193.08±6.22	20.46±7.05	8.36±0.18*	146.85±5.48b*	17.42±2.16
200	11.08±1.29**	221.35±4.50*	19.16±4.61	9.13±1.05*	161.30±6.23	17.08±0.93

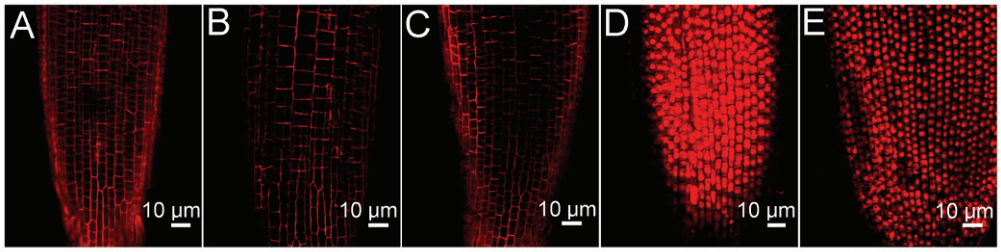

Fig. 2. Propidium iodide staining of the elongation zone in wheat roots under H_2O_2 treatment. *A* - control plants, *B* - 25 μM H_2O_2-treated plants, *C* - 50 μM H_2O_2-treated plants, *D* - 100 μM H_2O_2-treated plants, *E* - 200 μM H_2O_2-treated plants.

Table 3. Effects of 300 U dm^{-3} CAT and 200 μM BHT on H_2O_2-induced inhibition of root and shoot length [cm] in wheat seedlings. Means ± SE from at least three independent measurements. * and ** - significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively. $^{\Delta}$ - significantly different at $P < 0.05$ when compared with the H_2O_2 treatment alone.

Cultivar		Control	50 μM H_2O_2	50 μM H_2O_2 + CAT	50 μM H_2O_2 + BHT	200 μM H_2O_2	200 μM H_2O_2 + CAT	200 μM H_2O_2 + BHT
Ningchun	root length	4.89±0.55	1.33±0.39*	2.02±0.05$^{\Delta}$	1.25±0.87	0.37±0.15**	1.75±0.12$^{\Delta}$	1.93±0.28$^{\Delta}$
	shoot length	8.23±0.57	5.58±0.31*	5.30±0.62	4.93±0.35	4.23±0.29*	4.16±0.17	4.09±0.41
Xihan	root length	5.18±0.71	1.76±0.35*	3.51±0.21$^{\Delta}$	1.96±0.64	0.93±0.03**	1.84±0.07$^{\Delta}$	1.41±0.52$^{\Delta}$
	shoot length	9.31±0.45	7.72±0.45	8.30±0.48	7.79±0.62	5.38±0.32*	5.76±0.68	5.65±0.39

different H_2O_2 concentrations for 6 d, H_2O_2 fluorescent signal increased significantly and the maximal, strong fluorescence was observed in 200 μM H_2O_2-treated roots (Fig. 1*C*). Similarly, stronger H_2O_2 fluorescence was induced by H_2O_2 treatment in Xihan roots as compared with the control (Fig. 1*E, F*). These results indicated that exogenous H_2O_2 treatment resulted in the elevation of endogenous H_2O_2 content in plant root tissue.

MDA, the product of lipid peroxidation, is the symptom of oxidative stress in plants. In comparison with the control, root MDA content increased about 23, 22, 74, and 75 %, respectively, in Ningchun seedlings treated with 25, 50, 100, and 200 μM H_2O_2 for 6 d. In contrast, in Xihan seedlings, root MDA content did not significantly change under 25 μM H_2O_2 treatment, but increased to about 124, 132, and 144 % of the control value in response to 50, 100, and 200 μM H_2O_2, respectively (Table 2).

The application of 300 U dm^{-3} CAT together with H_2O_2 stimulated significantly ($P < 0.05$) the root growth in Ningchun and Xihan seedlings as compared with the H_2O_2 treatment alone. Also 200 μM BHT partly abolished inhibition of the root growth induced by 200 μM H_2O_2, but did not block the reduction of root length in response to 50 μM H_2O_2 treatment suggesting that the oxidative damage might be associated with the inhibition of root growth in wheat seedlings exposed to higher H_2O_2 concentration. However, these two agents could not reverse H_2O_2-inhibitory effect on the shoot growth (Table 3).

Fig. 2*A* shows long and narrow cell morphology with indistinct cell borders in root elongation region in untreated seedlings. In comparison with the control seedlings, exogenous H_2O_2 inhibited cell elongation in Ningchun root (Fig. 2*B,C,D,E* and Fig. 3). Similar changes of cell morphology in the root elongation zone in response to H_2O_2 treatment were observed in Xihan seedlings (data not shown). These results indicate that

Table 4. The changes of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) activities [U mg^{-1} protein] in leaves of two wheat cultivars after treatment with different H_2O_2 concentrations. Means ± SE from at least three independent measurements. * - significantly different from the control at $P < 0.05$.

Cultivar	H_2O_2 [μM]	SOD	CAT	POD	APX	GR
Ningchun	0	7.99±0.006	0.41±0.006	6.85± 0.01	0.49±0.02	11.08±0.72
	25	8.03±0.710	0.34±0.010*	5.43± 0.08*	0.55±0.02	10.55±1.22
	50	8.24±0.060*	0.32±0.022*	5.68± 0.11*	0.67±0.03*	11.38±1.44
	100	8.23±0.070*	0.33±0.011*	5.97± 0.14*	0.63±0.02*	11.41±0.91
	200	8.02±0.100	0.32±0.020*	6.09± 0.10*	0.69±0.02*	13.21±0.29
Xihan	0	5.26±0.015	0.06±0.001	194.42±15.44	1.23±0.07	7.20±0.60
	25	4.32±0.005	0.08±0.003	205.61± 6.26	1.35±0.05	7.23±0.48
	50	2.16±0.005*	0.18±0.013*	211.02±21.71	1.37±0.03	8.57±0.37*
	100	1.94±0.005*	0.11±0.005*	211.96±11.00	1.37±0.04	8.61±0.38*
	200	2.05±0.005*	0.18±0.014*	241.81±15.50	1.37±0.01	9.81±0.40*

H_2O_2 inhibited root cell elongation which might be reason for the inhibition of root growth and development observed in the two wheat cultivars. In addition, there was no detectable fluorescence in the nuclei of root cells exposed to lower H_2O_2 concentration (Fig. 2*B*,*C*) whereas the PI fluorescence (DNA content) of each nucleus in root cells was detected after exposure of the wheat seedlings to 100 and 200 μM H_2O_2 (Fig. 2*D*,*E*).

In comparison with the control, leaf SOD activity significantly increased in Ningchun treated with 50 and 100 μM H_2O_2 for 6 d, whereas H_2O_2 at 25 and 200 μM

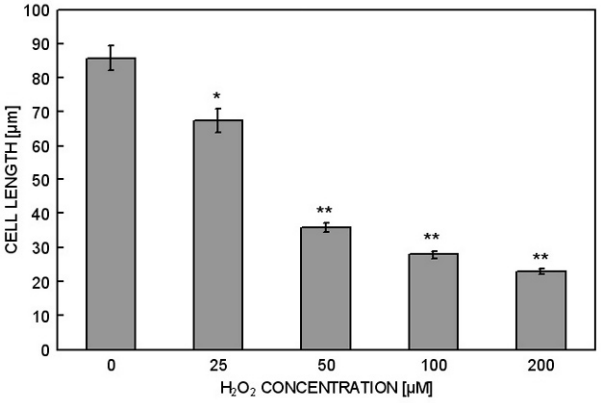

Fig. 3. Mean cell length in the elongation zone of a root tip corresponding to Fig 2*A*, *B*, *C*, *D,* and *E*. Values represent the means ± SE of at least 30 cells for each treatment (* and ** - significantly different at $P < 0.05$ and $P < 0.01$, respectively, when compared with the control).

had no effect on this enzyme. By contrast, 50, 100, and 200 μM H_2O_2 induced a significant decrease of leaf SOD activity in Xihan but an insignificant reduction in response to 25 μM H_2O_2 treatment (Table 4).

Leaf CAT activity was reduced to 82, 77, 84, and 79 % of the control value in Ningchun seedlings after treatment with 25, 50, 100, and 200 μM H_2O_2 for 6 d, respectively (Table 4). In contrast, leaf CAT activity enhanced 2.81, 1.78, and 2.83-folds in Xihan seedlings exposed to 50, 100 and 200 μM H_2O_2 treatment whereas 25 μM H_2O_2 induced an insignificant elevation in leaf CAT activity, as compared with the control seedlings.

Similarly, treatment of Ningchun seedlings with 25, 50, 100, and 200 μM H_2O_2 for 6 d resulted in about 21, 17, 13, and 11 %, respectively, decrease in leaf POD activity as compared with the control. However, no great difference in leaf POD activity was found between H_2O_2-treated and untreated Xihan seedlings (Table 4).

Compared with the control, leaf APX activity increased by 33, 27, and 38 %, respectively, in response to 50, 100, and 200 μM H_2O_2 treatment for 6 d. Apart from these, an insignificant increase (about 12 %) was detected in 25 μM H_2O_2-stressed plants. On the other hand, in Xihan seedlings treated with different H_2O_2 concentrations for 6 d, leaf APX activity remained almost unchanged in comparison with the untreated ones (Table 4).

Compared with the control, no significant changes in leaf GR activity were found in Ningchun seedlings treated with exogenous H_2O_2. Similarly, 25 μM H_2O_2 treatment had no effect on leaf GR activity in Xihan seedlings, but the application of other H_2O_2 concentration (50, 100, and 200 μM) resulted in a notable elevation in this enzyme activity.

A significant enhancement in leaf H_2O_2 content was observed in Ningchun exposed to exogenous H_2O_2 for 6 d in comparison with the control. However, leaf H_2O_2 content obviously decreased in Xihan treated with H_2O_2 (25, 50, and 100 μM) for 6 d and the lowest being at 25 μM H_2O_2. Additionally, no significant change in leaf MDA content was caused in these two wheat cultivars treated with different H_2O_2 concentrations for 6 d (Table 2).

Discussion

The increasing evidence shows that H_2O_2 treatment alone promotes seed germination of cereal plants such as barley, wheat, and rice (Ogawa and Iwabuchi 2001). Moreover, the ability of seed germination was reported to link to the accumulation of a critical level of H_2O_2 (Christophe 2008). However, *Lupinus albus* seeds are not sensitive to exogenous H_2O_2 (Cano *et al.* 1997). In the present study, two wheat cultivars showed different responses of seed germination to exogenous H_2O_2 treatment: 50 - 200 μM H_2O_2 treatment stimulated Ningchun seed germination but had no effect on Xihan seeds. Futher, we observed a significant reduction in growth parameters in both wheat cultivars under 25 to 200 μM H_2O_2 treatment but the growth of Ningchun seedlings was more sensitive to H_2O_2 stress than that of Xihan seedlings. One of the inevitable consequences of drought stress is enhanced ROS production including H_2O_2 in plants (Cruz de Carvalho 2008). Thus, we can suggest that drought sensitivity might be associated with the sensibility of Ningchun seedlings to H_2O_2. Additionally, the reduction of the root length was more prominent in the two wheat cultivars in comparison with the shoot length (Table 1) which could be explained by the fact that plant roots are the first point of contact with stress factors (Kabir *et al.* 2008). On the contrary, maybe due to the differences in the experimental design or plant species, the application of exogenous H_2O_2 promotes the formation and development of wheat and mung bean roots (He *et al.* 2009, Li *et al.* 2009).

At present, little is known about the mechanism of negative effects of H_2O_2 on plant growth. Exogenous CAT is a cell-impermeable scavenger of H_2O_2 (Karlsson *et al.* 2000). The application of CAT together with H_2O_2 promoted the root growth in the two wheat cultivars in comparison with the H_2O_2 treatment alone implying that the inhibition of the root growth in our experiments might be a consequence of increasing H_2O_2 content in H_2O_2-stressed Ningchun and Xihan seedlings. This was further supported by a significantly increased endogenous H_2O_2 content in H_2O_2-treated root tissue (Fig. 1). The study of Yamamoto *et al.* (2001) suggested that lipid peroxidation is an early symptom triggered by abiotic stress but not the primary cause of elongation inhibition in pea roots. However, in this study, the elevation of MDA content in response to H_2O_2 treatment was correlated with the reduction of root length in the wheat seedlings. Besides, antioxidant BHT could partly abolish 200 μM H_2O_2-induced inhibitory effect on the root growth, but did not affect 50 μM H_2O_2 treatment which suggested that the inhibition of root growth induced by higher H_2O_2 concentration might be associated with the strong oxidative damage. In agreement with our result, oxidative stress involved in the inhibition of plant growth was demonstrated by several studies (Singh *et al.* 2007, Upadhyay and Panda 2009).

The cell morphology in root elongation zone showed that the cell elongation was inhibited by increasing exogenous H_2O_2 concentration (Fig. 3). PI is a membrane-impermeant dye and is generally used to assay for the cell membrane permeability (Kennedy *et al.* 2011). There was no detectable fluorescence in the nuclei of root cells exposed to lower H_2O_2 concentration whereas the PI fluorescence of each nucleus in root cells was observed in 100 or 200 μM H_2O_2-treated seedlings indicating damage of the plasma membrane of the root cells under higher H_2O_2 concentration. On the basis of these findings, we could conclude that the cell elongation inhibition and the damage to the plasma membrane were closely linked to the reduction of root growth and development in wheat seedlings exposed to H_2O_2 treatment.

Plant responses to H_2O_2 treatment also include the changes of the redox status and the antioxidative response (Lin and Kao 2001). More recently, Zhang *et al.* (2011) reported that exogenous H_2O_2 stimulated antioxidant enzymes including SOD, APX, and GR in cucumber leaves. Similarily, elevated POD activity and oxidative stress tolerance were induced by exogenous H_2O_2 in *Cajanus cajan* (Goud and Kachole 2011). Our data showed different antioxidative responses in the two wheat cultivars leading to the increases of the SOD and APX activities but the decreases of the CAT and POD activities in Ningchun leaves, as well as the inhibition of SOD but the stimulation of CAT and GR in Xihan leaves. This could explain the fact that exogenous H_2O_2 treatment resulted in the enhancement and reduction in endogenous H_2O_2 content in the leaves of Ningchun and Xihan, respectively. Additionally, no significant difference in MDA content was observed in the leaves of untreated and H_2O_2-treated seedlings suggesting that the leaves of Xihan and Ningchun had a greater ability to eliminate ROS which was important in protecting the wheat seedlings from oxidative damage under different H_2O_2 concentrations.

In conclusion, exogenous H_2O_2 treatment stimulated the germination of Ningchun seeds but inhibited the root and shoot growth of the two wheat cultivars. Drought-sensitive Ningchun was more sensitive to H_2O_2 treatment than drought-tolerant Xihan. The increase of endogenous H_2O_2 content and the inhibition of cell elongation may be the cause of the reduction of root length under exogenous H_2O_2. In addition, the different antioxidative responses in the leaves of the two wheat cultivars might be responsible for higher tolerance of Xihan than Ningchun to H_2O_2 stress.

References

Aebi, H.: Catalase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Pp. 673-677. Academic Press, New York 1974.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Candan, N., Tarhan, L.: Tolerance or sensitivity responses of *Mentha pulegium* to osmotic and waterlogging stress in terms of antioxidant defense systems and membrane lipid peroxidation. - Environ. exp. Bot. **75**: 83-88, 2012.

Cano, A., Artes, F., Arnao, M.B., Sanchez-Bravo, J., Costa, M.A.: Influence of peroxides, ascorbate and glutathione on germination and growth in *Lupinus albus* L. - Biol. Plant. **39**: 457-461, 1997.

Christophe, B., Hayat, E.M.B., Françoise, C.: From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. - Compt. Rend. Biol. **331**: 806-814, 2008.

Cruz de Carvalho, H.M.: Drought stress and reactive oxygen species: production, scavenging and signaling. - Plant Signal Behav. **3**: 156-165, 2008.

Dhindsa, R.S., Matowe, W.: Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. - J. exp. Bot. **32**: 79-91, 1981.

Donahue, J.L., Okpodu, C.M., Cramer, C.L., Grabau, E.A., Alscher, R.G.: Responses of antioxidants to paraquat in pea leaves. - Plant. Physiol. **113**: 249-257, 1997.

Forman, H.J.: Use and abuse of exogenous H_2O_2 in studies of signal transduction. - Free Radical Biol. Med. **42**: 926-932, 2007.

Foyer, C.H., Lelandais, M., Kunert, K.J.: Photooxidative stress in plants. - Physiol. Plant. **92**: 696-717, 1994.

Fridovich, I.: Biological effects of the superoxide radical. - Arch. Biochem. Biophys. **247**: 1-11, 1986.

Gechev, T.S., Van Breusegem, F., Stone, J.M., Denev, I., Laloi, C.: Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. - Biol. Essays **28**: 1091-1101, 2006.

Gondim, F.A., Gomes-Filho, E., Lacerda, C.F., Prisco, J.T., Azevedo Neto, A.D., Marques, E.C.: Pretreatment with H_2O_2 in maize seeds: effects on germination and seedling acclimation to salt stress. - Braz. J. Plant Physiol. **22**: 103-112, 2010.

Goud, P.B., Kachole, M.S.: Effect of exogenous hydrogen on peroxide and polyphenol oxidase in *Cajanus cajan* (L.) Millsp. detached leaves. - Int. J. Current Res. **3**: 61-65, 2011.

He, L.H., Gao, Z.Q., Li, R.Z.: Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. - African J. Biotechnol. **8**: 6151-6157, 2009.

Kabir, M., Zafar Iqbal, M., Shafiq, M., Farooqi, Z.R.: Reduction in germination and seedling growth of *Thespesia populnea* L., caused by lead and cadmium treatments. - Pakistan J. Bot. **40:** 2419-2426, 2008.

Karlsson, A., Nixon, J.B., McPhail, L.C.: Phorbol myristate acetate induces neutrophil NADPH-oxidase activity by two separate signal transduction pathways: dependent or independent of phosphatidylinositol 3-kinase. - J. Leuk. Biol. **67**: 396-404, 2000.

Karuppanapandian, T., Moon, J.C., Kim, C., Manoharan, K., Kim, W.: Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. - Aust. J. Crop Sci. **5**: 709-725, 2011.

Kennedy, D., Cronin, U.P., Wilkinson, M.G.: Responses of *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* to simulated food processing treatments, determined using fluorescence-activated cell sorting and plate counting. - Appl. environ. Microbiol. **7**: 4657-4668, 2011.

Li, J.T., Qiu, Z.B., Zhang, X.W., Wang, L.S.: Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. - Acta Physiol. Plant. **30**: 835-842, 2011.

Li, S.W., Xue, L.G., Xu, S.J., An, L.Z.: Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings. - Environ. exp. Bot. **65**: 63-71, 2009.

Lin, C.C., Kao, C.H.: Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings. - Plant Soil **230**: 135-143, 2001.

Liu, Y., Wan, Q., Wu, R., Wang, X., Wang, H., Wang, Z., Shi C., Bi, Y.: Role of hydrogen peroxide in regulating glucose-6-phosphate dehydrogenase activity under salt stress. - Biol. Plant. **56**: 313-320, 2012.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. **7**: 405-410, 2002.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Ogawa, K., Iwabuchi, M.: A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. - Plant Cell Physiol. **42**: 286-291, 2001.

Ozden, M., Demirel, U., Kahraman, A.: Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. - Sci. Hort. **119**: 163-168, 2009.

Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G., Grill, E., Schroeder, J.I.: Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. - Nature **406**: 731-734, 2000.

Peng, L.T., Yang, S.Z., Li, Q., Jiang, Y.M., Joyce, D.C.: Hydrogen peroxide treatments inhibit the browning of fresh-cut Chinese water chestnut. - Post Biol. Technol. **47**: 260-266, 2008.

Rao, M.V., Paliyath, G., Ormrod, D.P.: Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis* thaliana. - Plant Physiol. **110**: 125-136, 1996.

Sergiev, I., Alexieva, V., Karanov, E.: Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. - Compt. Rend. Acad. Bulg. Sci. **51**: 121-124, 1997.

Singh, H.P., Batish, D.R., Kohli, R.K., Arora, K.: Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. - Plant Growth Regul. **53**: 65-73, 2007.

Upadhyay, R.K., Panda, S.K.: Copper-induced growth inhibition, oxidative stress and ultrastructural alterations in freshly grown water lettuce (*Pistia stratiotes* L.). - Compt. Rend. Biol. **332**: 623-32, 2009.

Yamamoto, Y., Kobayashi, Y., Matsumoto, H.: Lipid

peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. - Plant Physiol. **125**: 199-208, 2001.

Yang, Y.L., Zhang, Y.Y., Lu, J., Zhang, H., Liu, Y., Jiang, Y., Shi, R.X.: Exogenous H_2O_2 treatment induced antioxidative responses and the signal regulation of proline accumulation in halophyte *Nitraria tangutorum* Bobr. callus. - Biol. Plant. **56**: 330-336, 2012.

Zhang, X.L., Jia, X.F., Yu, B., Gao, Y., Bai, J.G.: Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. - Sci. Hort. **129**: 656-662, 2011.

Zhou, Q.: The measurement of malondialdehyde in plants. - In: Zhou, Q.(ed): Methods in Plant physiology. Pp. 72-74. China Agricultural Press, Beijing 2001.