# Role of hydrogen peroxide in regulating glucose-6-phosphate dehydrogenase activity under salt stress

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## Abstract

The role of hydrogen peroxide in the regulation of glucose-6-phosphate dehydrogenase (G6PDH) activity in the red kidney bean (*Phaseolus vulgaris* L.) roots under salt stress (100 mM NaCl) was investigated. Salt stress caused the increase of the activities of G6PDH and antioxidative enzymes including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), as well as $H_2O_2$ production. The application of $H_2O_2$ (1 mM) also enhanced the activities of G6PDH as well as antioxidative enzymes. In the presence of exogenous CAT, $H_2O_2$ content was decreased, and the enhanced activities of G6PDH and antioxidative enzymes induced by NaCl or by exogenous $H_2O_2$ were also abolished, suggesting that the enhancement of the above enzyme activities under salt stress was a result of the increased endogenous $H_2O_2$ levels. Further results showed that the effects of NaCl and $H_2O_2$ on the activities of antioxidative enzymes were diminished by $Na_3PO_4$ (a G6PDH inhibitor), suggesting G6PDH activity is required in enhancing the activities of antioxidative enzymes. The enhanced membrane leakage, lipid peroxidation, $H_2O_2$ and $O_2^-$ contents, G6PDH and antioxidative enzyme activities under salt stress were all recovered to control level when the red kidney bean seedlings treated with 100 mM NaCl for 6 d were transferred to the control conditions for 8 d.

*Additional key words*: antioxidative enzymes, NaCl, *Phaseolus vulgaris*, red kidney bean, redox status.

## Introduction

Salinity is one of the major abiotic stresses affecting plant productivity due to its negative effects on plant growth, ion balance and water relations (Hasegawa *et al.* 2000, Maia *et al.* 2010). Higher plants have evolved multiple protective mechanisms against salt stress including ion homeostasis, osmolyte production, reactive oxygen species (ROS) scavenging, *etc.* (Hasegawa *et al.* 2000, Fedina *et al.* 2009, Mutlu *et al.* 2009, Maia *et al.* 2010).

Hydrogen peroxide participates in plant metabolism (*e.g.*, cell wall biosynthesis) and also plays a role in numerous stress signaling pathways, pathogen response, the programmed cell death or hormonal action (Hernández *et al*. 1993, Dat *et al*. 2000, Mittler 2002, Mullineaux and Karpinski 2002, Apel and Hirt 2004, Foyer and Noctor 2005b, Fedina *et al.* 2009). However, $H_2O_2$ can also be converted to the dangerous hydroxyl radical *via* Fenton reaction, which can cause lipid peroxidation, protein degradation and DNA damage (Fridovich 1986). Failure to control ROS accumulation may lead to the phenomenon known as oxidative stress (Bartosz 1997, Foyer and Noctor 2000).

Glucose-6-phosphate dehydrogenase (G6PDH) is the key regulatory enzyme in the pentose phosphate pathway. In tobacco cells, oxidative stress was induced by an elicitor stimulated G6PDH activity (Pugin *et al.* 1997, Yu *et al.* 2004). Moreover, exposure of parsley cells to UV radiation resulted in an increased transcription rate for *G6PDH* gene (Logemanm *et al*. 2000). Some reports also

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*Abbreviations*: APX - ascorbate peroxidase; CAT - catalase; G6PDH - glucose-6-phosphate dehydrogenase; MDA - malondialdehyde; 6PGD - 6-phosphogluconate dehydrogenase; POD - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive substances.
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indicated that salt stress could increase G6PDH activity in plants (Nemoto and Sasakuma 2000, Liu *et al*. 2007). However, the regulative mechanisms of G6PDH in plant tolerance to oxidative stress are still unclear.

Cellular redox status affects many physiological processes in plants, such as apoptosis (Cai and Jones 1999), oxidative defense (Foyer and Noctor 2005b), senescence (Groten *et al*. 2005), allosteric control of enzyme activities, transcription and translation (Apel and Hirt 2004), and a variety of signal transduction events (Neill *et al*. 2002). However, environmental stresses inevitably induce the production of ROS (Hernández *et al*. 1995, Hasegawa *et al*. 2000). A balance exists in cells between the ROS generation and scavenging (Dutilleul *et al.* 2003). ROS could be scavenged by both enzymatic and non-enzymatic antioxidative pathways (Song *et al.* 2006, Zhang *et al*. 2007, Mutlu *et al.* 2009, Maia *et al.* 2010, Vuletić *et al.* 2010). This complex network of pro- and antioxidative system governs the "redox homeostasis" inside the cell. The object of the present study is to elucidate whether the G6PDH activity is involved in the re-establishment of redox status under salt stress, and the roles of $H_2O_2$ in the process.

## Materials and methods

Red kidney bean (*Phaseolus vulgaris* L.) seeds were purchased from *Lanzhou Grain Supply Center*, Xincun, China. Seeds were imbibed in water for 24 h then seed coats were peeled. Seeds were incubated at 25 °C for 24 h to germinate. Germinated seeds were bedded in quartzite and watered with 25 % Hoagland solution. Plants were kept at 14-h photoperiod with a photosynthetically active radiation (PAR) of 120 μmol $m^{-2}$ $s^{-1}$ and temperature of 25 °C and for 6 d. All treatments started on the 7$^{th}$ day.

Hoagland medium was supplemented with different concentrations of NaCl (salt stress; Hilal *et.al*. 1998). CAT (*Sigma*, St. Louis, USA) at 100 U $cm^{-3}$ was used to eliminate $H_2O_2$ and $Na_3PO_4$ (2.5 mM) was used as an inhibitor of G6PDH (Liu *et al.* 2007). The control was treated with only 25 % Hoagland medium. All treatments were applied for 24 h.

$H_2O_2$ assay was carried out according to the method described by Frew *et al*. (1983). The red kidney bean roots (0.5 g) were extracted by 3 $cm^3$ cold acetone and extracts were centrifuged at 5 000 *g* at 4 °C for 10 min. 1 $cm^3$ of the supernatant was mixed with 0.1 $cm^3$ 5 % $TiSO_4$ and 0.1 $cm^3$ ammonia. After centrifugation at 3 000 *g* for 10 min, the pellet was resuspended in 4 $cm^3$ 2 M $H_2SO_4$. $H_2O_2$ contents were determined at 415 nm with a spectrophotometer (*DU640, Beckman*, USA).

Superoxide contents were measured as described by Elstner and Heupel (1976). 0.5 g of roots was ground and extracted in 2 $cm^3$ 65 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 5 000 *g* for 10 min at 4 °C. 1 $cm^3$ of the supernatant was mixed with 0.9 $cm^3$ 65 mM phosphate buffer (pH 7.8) and 0.1 $cm^3$ 10 mM hydroxylamine hydrochloride, and then the mixture was incubated at 25 °C for 20 min. After that, 1 $cm^3$ of the mixture, 1 $cm^3$ 17 mM anhydrous *p*-aminobenzene sulfonic acid and 1 $cm^3$ 17 mM 1-naphthylamine were mixed, and then incubated at 25 °C for 20 min. The absorbance was monitored at 530 nm after 3 $cm^3$ *n*-butyl alcohol was added to the mixture.

The G6PDH was extracted according to the method described by Esposito *et al*. (2001). The crude extract was used to determine enzyme activation. 0.1 $cm^3$ extract was added to the total dehydrogenase assay buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, 0.2 mM glucose 6-phosphate, 0.2 mM 6-phosphogluconate, and 0.1 mM NADP, pH 8.1 (all reagents from *Sigma*) and the 6-phosphogluconate dehydrogenase (6PGD) assay buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, 0.2 mM 6-phosphogluconate, and 0.1 mM NADP, pH 8.1). NADP reduction to NADPH was measured as the rate of change of the absorbance at 340 nm over 6 min. G6PDH activity was calculated as the total dehydrogenase activity minus the 6PGD activity (Tian *et al*. 1998).

For enzyme extraction, 0.5 g of roots were homogenized with a mortar and pestle in 3 $cm^3$ of ice-cold 50 mM K-phosphate buffer, pH 7.8, 1 mM EDTA containing 5 mM Cys, 1% (m/v) polyvinyl pyrrolidone. For APX activity, 20 mM sodium ASC was added. The homogenate was filtered through one layer of *Miracloth* (*Calbiochem*, USA) and centrifuged at 15 000 *g* for 20 min. The supernatant was used for enzyme determination. Catalase activity (CAT, EC 1.11.1.6) was assayed by following the decomposition of $H_2O_2$ at 240 nm (Aebi 1984). Ascorbate peroxidase activity (APX, EC 1.11.1.11) was measured in the presence of 0.25 mM ascorbic acid and 0.5 mM $H_2O_2$ by monitoring the decrease in absorption at 290 nm (Janda *et al*. 1999). Superoxide dismutase activity (SOD, EC 1.15.1.1) was determined as described by Kruse *et al.* (1995). One unit of SOD was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of nitroblue tetrazolium (NBT) as monitored at 560 nm. Peroxidase activity (POD, EC 1.11.1.7) was determined according to Adam *et al.* (1995) by monitoring the rate of guaiacol oxidation at 470 nm ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$). All enzymes activities were measured at 25 °C in 3 $cm^3$ reaction mixtures. Measurements were made with a spectro-photometer (*DU640*, *Beckman*) with no lag period.

The occurrence of malondialdehyde (MDA), a secondary end product of the oxidation of polyunsa-turated fatty acids, is considered a useful index of lipid

peroxidation. MDA can be measure by determining the thiobarbituric acid-reactive-substances (TBARS) according to the method described by Hodges *et al*. (1999).

Membrane permeability (MP) was determined according to Sairam and Srivastava (2002) with some modifications. The roots (0.1 g) were placed in cuvette with 10 $cm^3$ of de-ionized water at 25 °C for 3 h. After the incubation, the conductivity in the bathing solution was determined (C1). Then, the samples were heated at 90 °C for 1 h, and conductivity was read again in the bathing solution (C2). Electrolyte leakage was expressed as a percentage of the total conductivity after heating at 90 °C (MP = C1/C2 × 100).

SDS-PAGE was performed as described by Laemmli (1970). Proteins (5 μg) were solubilized and separated on a 10 % (m/v) acrylamide gel. After electrophoresis, the separated proteins were transferred to a nitrocellulose membrane. The membrane was blocked for 60 min with 5 % (m/v) nonfat milk in 0.05 % (m/v) *Tween 20*, 10 mM Tris (pH 8.0), and 150 mM NaCl. A polyclonal antibody raised against G6PDH (*Sigma*) was added and incubated with the membrane overnight. After washing, the alkaline phosphatase-coupled secondary antibody was added and incubated for 1.5 h. The colour was developed with a solution containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Each experiment was repeated at least three times. An *ANOVA* test followed by a rank test was performed. In all cases, the confidence coefficient was set at 0.05.

## Results

$H_2O_2$ contents increased slightly in the red kidney bean roots at the lower concentrations (50 - 100 mM) of NaCl. When NaCl concentrations were increased from 100 mM to 200 mM, $H_2O_2$ contents increased sharply to 133.6 and 185.7 % of the control, respectively (Table 1). However, in the presence of 400 mM NaCl, $H_2O_2$ contents decreased to a very low level. G6PDH activity also increased with the increasing NaCl concentrations and maximum G6PDH activity (166.8 % of the control) was reached at 100 mM NaCl. But it decreased rapidly when concentration was higher than 100 mM (Table 1).

Under the exogenous 1 mM $H_2O_2$ treatment, G6PDH activity was enhanced (Table 2). It increased to about 173.9 % of the control after 1 mM $H_2O_2$ treatment for 24 h. However, G6PDH activity decreased significantly in the presence of higher concentrations (5 - 10 mM) of $H_2O_2$. So 1 mM $H_2O_2$ was used in the following experiments. To further understand the relationship between $H_2O_2$ and the elevated G6PDH activity under salt stress, CAT, a scavenger of $H_2O_2$, was used. NaCl-induced $H_2O_2$ accumulation was effectively reversed by application of CAT. After that, G6PDH activity decreased to 74.1 % of the control. The CAT treatment alone also induced the decrease of G6PDH activity (62.5 % of the control; Table 3).

Table 1. Effects of salt stress on $H_2O_2$ content [μmol $g^{-1}$ (d.m.)] and G6PDH activity [nmol(NADPH) $mg^{-1}$(prot.) $min^{-1}$]. The red kidney bean seedlings were cultured in different concentrations of NaCl for 24 h. Means ± SE of three independent experiments. Means with different letters were significantly different at the 0.05 level.

| NaCl [mM] | $H_2O_2$ | G6PDH |
|---|---|---|
| 0 | 24.4 ± 1.9 $^{a}$ | 280.8 ± 10.4 $^{a}$ |
| 50 | 29.6 ± 1.8 $^{b}$ | 321.5 ± 20.8 $^{b}$ |
| 100 | 32.6 ± 1.3 $^{b}$ | 468.5 ± 17.5 $^{c}$ |
| 150 | 45.3 ± 0.9 $^{c}$ | 247.3 ± 20.6 $^{d}$ |
| 200 | 50.9 ± 3.6 $^{d}$ | 230.6 ± 12.3 $^{d}$ |
| 400 | 19.2 ± 0.8 $^{e}$ | 130.7 ± 9.5 $^{e}$ |

The antioxidative enzyme activities in the red kidney bean roots increased significantly in salt stress. The activities of APX, POD, SOD and CAT increased to 141.5, 151.7, 233.3 and 19.7 % of the control, respectively. Thus, we observed the role of G6PDH in the regulation of the antioxidative enzyme activities under salt stress. So $Na_3PO_4$, an effective inhibitor of G6PDH (Shen and Chen 1959, Bi and Liang 1987, Liu *et al.*

Table 2. Time course of G6PDH activity [nmol(NADPH) $mg^{-1}$(prot.) $min^{-1}$] under 1 - 10 mM $H_2O_2$ treatments.

| $H_2O_2$ [mM] | 0 h | 3 h | 6 h | 12 h | 24 h |
|---|---|---|---|---|---|
| 0 | 298.1 ± 12.6 $^{a}$ | 307.5 ± 15.7 $^{bf}$ | 309.7 ± 10.8 $^{b}$ | 321.7 ± 20.4 $^{bf}$ | 311.4 ± 18.96 $^{bf}$ |
| 1 | 298.1 ± 12.6 $^{a}$ | 315.6 ± 17.8 $^{bf}$ | 335.8 ± 15.6 $^{f}$ | 424.1 ± 27.3 $^{g}$ | 515.8 ± 30.4 $^{h}$ |
| 5 | 298.1 ± 12.6 $^{a}$ | 191.4 ± 13.5 $^{cd}$ | 224.6 ± 11.6 $^{d}$ | 219.7 ± 23.5 $^{d}$ | 218.7 ± 15.7 $^{d}$ |
| 10 | 298.1 ± 12.6 $^{a}$ | 178.9 ± 15.4 $^{c}$ | 168.6 ± 10.1 $^{c}$ | 143.6 ± 15.3 $^{ce}$ | 145.8 ± 9.5 $^{e}$ |

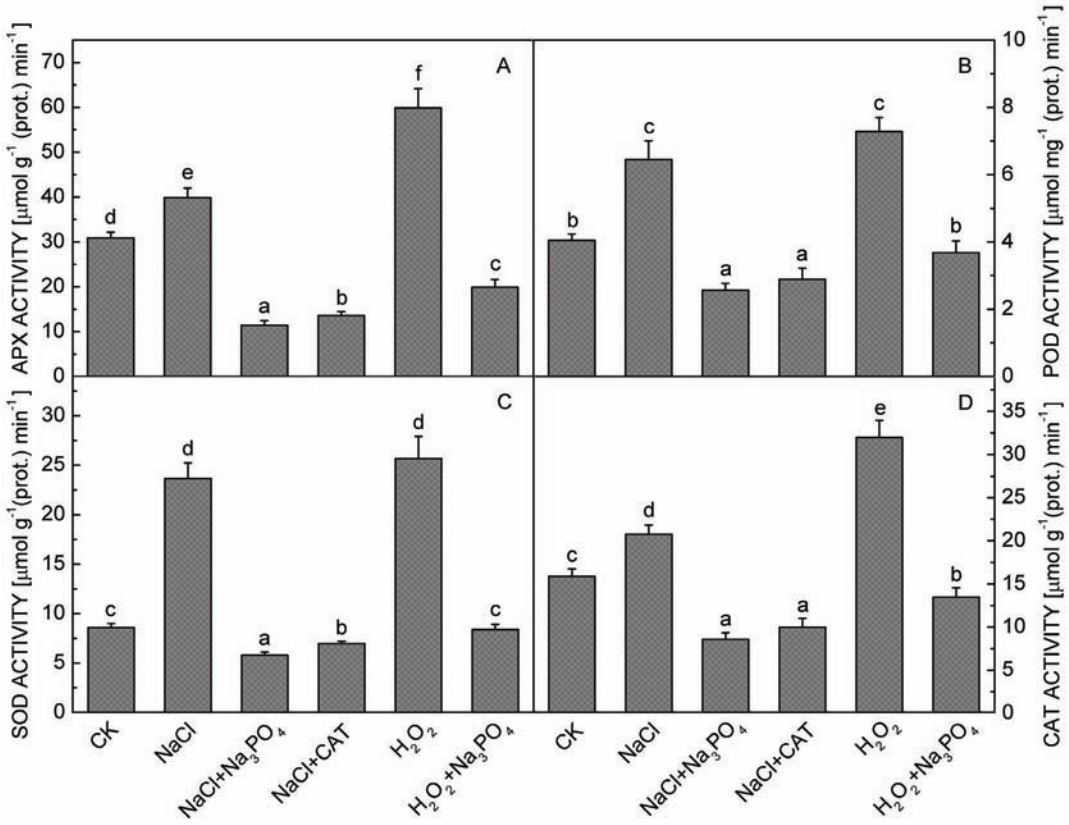


Fig. 1. NaCl-induced activity increase of APX (*A*), POD (*B*), SOD (*C*) and CAT (*D*) requires G6PDH and $H_2O_2$ in the red kidney bean roots. 100 mM NaCl, 1 mM $H_2O_2$, 2.5 mM $Na_3PO_4$ or 100 U $cm^{-3}$ CAT were contained in the 25 % Hoagland medium for treatments for 24 h. Means ± SE of three independent experiments. Within each set of experiments, *bars* with different letters were significantly different at the 0.05 level.

2007), was used in the study. Under NaCl + $Na_3PO_4$ treatment, the activities of APX, POD, SOD and CAT were reduced significantly by 63.1, 36.8, 32.6 and 46.0 %, respectively, compared with the control (Fig. 1).

The exogenous $H_2O_2$ increased significantly the antioxidative enzyme activities. The activities of APX, POD, SOD and CAT increased to 194.0, 179.8, 299.5 and 201.5 % of the control, respectively (Fig. 1). When the NaCl-induced $H_2O_2$ accumulation was abolished under NaCl + CAT treatment, their activities decreased to the lower levels. The antioxidative enzyme activities also decreased significantly under $H_2O_2$ + $Na_3PO_4$ treatment (Fig. 1).

Table 3. Effects of NaCl, exogenous $H_2O_2$ and CAT on endogenous $H_2O_2$ [μmol $g^{-1}$ (d.m.)] and G6PDH activity [nmol(NADPH) $mg^{-1}$(prot.) $min^{-1}$]. The seedlings were incubated in control solution (CK) or in 100 mM NaCl solution in the absence or presence 1 mM $H_2O_2$ or 100 U $cm^{-3}$ CAT for 24 h.

| Treatments | $H_2O_2$ | G6PDH |
|---|---|---|
| CK | 21.8 ± 1.9 $^{b}$ | 297.9 ± 12.7 $^{c}$ |
| NaCl+$H_2O_2$ | 42.7 ± 3.5 $^{d}$ | 562.4 ± 24.2 $^{d}$ |
| CAT | 10.4 ± 1.2 $^{a}$ | 186.3 ± 10.4 $^{a}$ |
| NaCl | 35.5 ± 2.8 $^{c}$ | 531.3 ± 15.0 $^{d}$ |
| NaCl+CAT | 11.3 ± 1.5 $^{a}$ | 220.8 ± 9.2 $^{b}$ |

A new balance of redox status was formed in the red kidney bean roots under salt stress (Tables 2 and 3). In the control, the antioxidative enzyme activities (APX, POD and CAT) and the ROS contents ($H_2O_2$ and $O_2^{\cdot-}$) increased steadily during the cultivation period with the exception of SOD activity. However, the antioxidative enzyme activities and the ROS contents increased rapidly from day 0 to day 6 under salt stress and then decreased. After 10 d, both antioxidative enzyme activities and ROS contents remain constant under salt stress. The antioxidative enzyme activities and ROS contents decreased the original levels when the red kidney bean roots treated by 100 mM NaCl for 6 d were transferred to the control conditions. Similar patterns were observed in membrane permeability (MP) and TBARS contents as well as G6PDH activity. Nevertheless, the antioxidative enzyme activities, ROS contents, G6PDH activity as well as the levels of MP and TBARS showed the higher levels in the continuous salt stress compared with those in control.

To understand the mechanism of the increased

G6PDH activity under salt or $H_2O_2$ treatment, the levels of G6PDH protein were examined in the red kidney bean roots. Western-blot analysis demonstrated that the content of G6PDH protein increased under NaCl or $H_2O_2$ treatments. It decreased in the presence of $H_2O_2$ scavenger (CAT) in both control and salt stressed plants (Fig. 2). These results indicated that the salt stress induced increase in G6PDH expression might be related to the $H_2O_2$ release.

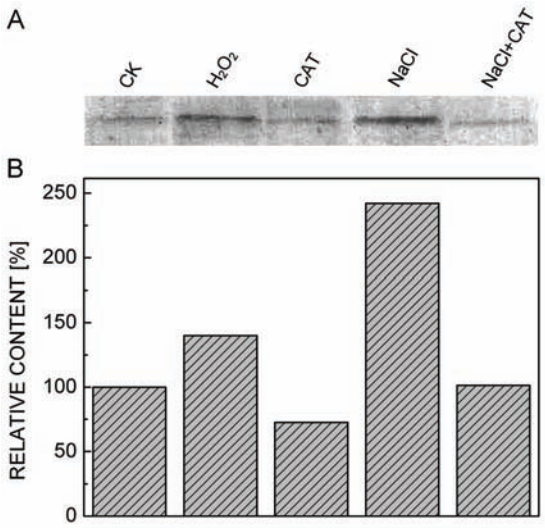


Fig. 2. Western-blot analysis of G6PDH expression in the red kidney bean roots under salt stress. The equal amounts of total proteins were loaded in each lane. The red kidney bean seedlings were treated as described in Fig. 1. Three independent experiments were performed and one representative immunoblotting result was shown.

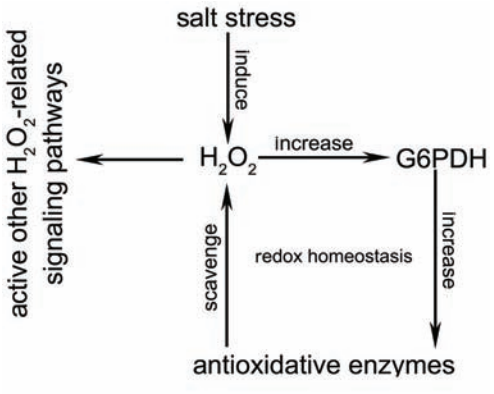


Fig. 3. The schematic model for the involvement of $H_2O_2$-dependent G6PDH activity in maintaining the intracellular redox homeostasis under salt stress in the red kidney roots. Under salt stress, the elevated $H_2O_2$ level stimulated G6PDH activity and expression, which was finally involved in the process of the $H_2O_2$-induced antioxidative enzyme activities. This enhanced antioxidative ability could facilitate to keep $H_2O_2$ in a steady state for signal. During this process, $H_2O_2$ and G6PDH play an important role in regulating cellular redox state under salt stress.

## Discussion

The involvement of G6PDH in stress responses has been demonstrated in several studies including oxidative stress (Hauschild and Schaewen 2003, Debnam *et al*. 2004), pathogenesis (Tecsi *et al*. 1994, Batz *et al*. 1998, Šindelář and Šindelářová 2002), salt stress (Nemoto and Sasakuma 2000), and metal toxicity (Esposito *et al*. 1998). We have previously reported that G6PDH was involved in the resistance to oxidative stress induced by salt stress (Liu *et al*. 2007, Wang *et al*. 2008a, b). In this study, the results showed that $H_2O_2$ contents and G6PDH activity increased in red kidney bean roots under low NaCl concentration (50 - 100 mM; Table 1). The exogenous $H_2O_2$ (1 mM) also enhanced significantly G6PDH activity (Table 2). The effect of $H_2O_2$ on G6PDH activity under salt stress was further tested by using $H_2O_2$ scavenger CAT. In the presence of CAT, $H_2O_2$ content was reduced in both control and NaCl stressed plants, and the NaCl- or $H_2O_2$-induced G6PDH activity was also abolished. These results suggested that $H_2O_2$ may act as a signal in the enhancement of G6PDH activity under salt stress in the red kidney bean roots.

Foyer and Noctor (2005a) suggested that the accumulation of ROS must be controlled but ROS are also able to act as signaling molecules. The $H_2O_2$ signaling mechanisms include the direct effects of $H_2O_2$ on proteins, particularly on transcription factors, which result in changes in gene expression (Davletova *et al.* 2005, Foyer and Noctor 2005a). In this study, Western blot further showed that the G6PDH protein contents in the red kidney bean roots increased in the presence of 100 mM NaCl or 1 mM $H_2O_2$ (Fig. 4). The application of CAT reversed effectively the enhanced G6PDH protein contents induced by NaCl or $H_2O_2$. Davletova *et al.* (2005) also reported that the heat shock response could be completely inhibited by effective removal of $H_2O_2$, because expression of genes such as those encoding HSF21 and HSF5 and cytosolic APX1 was modulated by ROS signals. Thus, we concluded that the elevation of G6PDH activity in NaCl or $H_2O_2$ treated roots resulted from the enhanced G6PDH protein expression. These results supported a signal role of $H_2O_2$ in the enhancement of G6PDH activity under NaCl stress, and also clarified its mechanism in the process.

It has been indicated that NaCl stress induced ROS accumulation (Hernández *et al*. 1993, 1994) and activated antioxidative enzymes such as CAT, SOD, GR, APX and POD (Liu *et al*. 2007, Xing *et al*. 2007, Wang *et al.* 2008a,b, Mutlu *et al.* 2009, Maia *et al.* 2010). In present study, the antioxidative enzymes could be activated by both 100 mM NaCl and 1 mM $H_2O_2$. The activation

effect of NaCl on antioxidative enzyme activities could be eliminated by CAT ($H_2O_2$ scavenger), suggesting that $H_2O_2$ played a crucial role in NaCl stress through enhancing the antioxidative enzyme activities (Fig. 1). Zhang *et al*. (2006) reported $H_2O_2$ involvement in the stress-activated antioxidative enzymes. Thus, salt stress induced $H_2O_2$ accumulation and the enhanced $H_2O_2$ contents further stimulated the antioxidative enzyme activities, which in turn avoided the excessive accumulation of ROS under salt stress.

The NaCl- or $H_2O_2$-induced antioxidative enzyme activities could be effectively abolished when G6PDH activity was inhibited by $Na_3PO_4$ in the red kidney bean roots (Fig. 1). Based on these results, we suggested that G6PDH was involved in the process of the $H_2O_2$-regulated antioxidative enzyme activities under salt stress. Thus, G6PDH increased the intracellular anti-oxidative enzyme activities, which in turn decreased the levels of ROS.

Growing evidence suggests a model for redox homeostasis in which the ROS-antioxidative enzymes interaction acts as an interface for signals derived from metabolism and from the environment (Foyer and Noctor 2005a). For further elucidation of the roles of $H_2O_2$ and G6PDH in regulating the intracellular redox state, both the continuous NaCl treatment and recovery experiments were performed. When the red kidney bean roots after 6 d treatment with 100 mM NaCl were transferred to the control conditions, antioxidative enzyme activities, ROS contents, G6PDH activity as well as the levels of MP and TBARS decreased to the control levels. The recovery suggested that the red kidney bean roots could endure 100 mM NaCl stress. Under the continuous NaCl treatment, the above parameters increased significantly at the first 6-d treatments, and remained constant thereafter. However, the contents of antioxidative enzymes and ROS were higher under continuous salt stress compared with those in control (Figs. 2 and 3). G6PDH activity showed a similar profile. Our results confirmed that $H_2O_2$ and G6PDH were involved in the NaCl-induced antioxidative enzyme activities in the red kidney bean roots. Thus, we concluded that $H_2O_2$ and G6PDH played an important role in regulating the intracellular redox homeostasis under salt stress.

In conclusion, our results showed that salt stress induced $H_2O_2$ accumulation, and $H_2O_2$, as a signal, stimulated G6PDH activity as well as its protein expression under NaCl stress in the red kidney bean roots. The enhanced G6PDH activity was finally involved in the process of the $H_2O_2$-induced increase in antioxidative enzyme activities under salt stress. Thus, the enhanced antioxidant ability can facilitate the maintenance of a steady-state level of $H_2O_2$ in cells, and allows the beneficial functions of $H_2O_2$ for signaling as well as a defensive response to salt stress. During this process, $H_2O_2$ and G6PDH play a central role in the tolerance of salt stress in red kidney bean roots.

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