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Heat shock pretreatment suppresses cadmium-induced ammonium ion accumulation and phenylalanine ammonia-lyase activity in rice seedling leaves

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ABSTRACT. We investigated the effects of heat shock (HS) on the subsequent cadmium (Cd)-induced ammonium ion (HN_4^+) accumulation and the phenylalanine ammonia-lyase (PAL) activity in rice seedling leaves. Increases in PAL activity occurred prior to NH_4^+ increases in rice leaves. Both of these Cd-induced increases were significantly deterred by the potent PAL inhibitor α-aminooxy-β-phenylpropionic acid. Exposing rice seedlings to 3 h HS in the dark effectively reduced subsequent Cd-induced increases in PAL activity and NH_4^+ content. The HS effect can be mimicked by pretreating rice seedlings with exogenous H_2O_2, glutathione (GSH), ascorbic acid (AsA), or L-galactono-1,4-lactone (GalL, a precursor of AsA biosynthesis) under non-HS conditions. The protection that HS provides however, can be counteracted by imidazole, a NADPH oxidase inhibitor, buthionine sulfoximine (BSO, a GSH synthesis inhibitor), or lycorine (Lyc, an AsA synthesis inhibitor). Furthermore, the effects of BSO and Lyc can be reversed by the addition of GSH and AsA, respectively. The mechanisms of the protective effect of HS against subsequent Cd effects are discussed.

Keywords: Ammonium ion; Cadmium; Heat shock; *Oryza sativa* L.; Oxidative stress; Phenylalanine ammonia-lyase.

Abbreviation: AOPP, α-aminooxy-β-phenylpropionic acid; **AsA,** ascorbic acid; **BSO,** buthionine sulfoximine; **GalL,** L-galactono-1,4-lactone; **HS,** heat shock; **hsps,** heat shock proteins; **IMD,** imidazole; **Lyc,** lycorine; **PAL,** phenylalanine ammonia-lyase; **ROS,** reactive oxygen species.

INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals. Although naturally occurring amounts of Cd are generally low, anthropogenic activities can significantly increase its concentration (Gratao et al., 2005). Plants take up excess Cd from the soil, which has a direct or indirect effect on physiological processes such as respiration, photosynthesis, cell elongation, plant-water relationships, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (Sanitá di Toppi and Gabbrielli, 1999).

In both prokaryotic and eukaryotic cells, oxygen activation is a general phenomenon leading to the production of O_2^-, H_2O_2, and OH· (reactive oxygen species, ROS) by a step-wise one-electron transfer to molecular oxygen (Gechev et al., 2006). ROS are formed in normal cell metabolism and their level increases under stress conditions. Similar to other organisms, plants have developed protection systems, either constitutive or inducible, to counteract oxidative stress (Gechev et al., 2006). Several lines of evidence show that oxidative stress is a major component of Cd stress (Cho and Seo, 2005; Gratao et al., 2005; Hsu and Kao, 2007).

The ammonium ion (NH_4^+) is a central intermediate of nitrogen metabolism in higher plants (Miflin and Lea, 1976). A high content of NH_4^+ has a toxic effect on plant cells (Givan, 1979). When exposured to Cd, NH_4^+ levels increase in rice leaves (Chien and Kao, 2000; Hsu and Kao, 2003; Hsu et al., 2006). Chien et al. (2002) also found that the accumulation of NH_4^+ in rice leaves is a consequence of the oxidative damage caused by Cd.

Phenylalanine ammonia-lyase (PAL) is the first enzyme in phenylpropanoid metabolism (Hahlbrock and Scheel, 1989), catalyzes the elimination of NH_4^+ from phenylalanine, and produces *trans*-cinnamate (Hahlbrock and Grisebach, 1979). Treatment with $CdCl_2$ results in an increase in PAL activity in rice leaves (Hsu and Kao, 2004). Kumar and Knowles (2003) demonstrated that wounding-induced PAL activity is related to the ability to produce superoxide radicals in potato tuber.

Exposing plants to temperatures 5 to 15°C above the normal growing conditions for 15 min to a few hours is usually considered heat shock (HS) treatment. Wheat

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leaf segments exhibited an acquired protection against Cd and other heavy metal-reduced cell viability following seedling exposure to HS in the dark (Orzech and Burke, 1988). Neumann et al. (1994) demonstrated that HS treatment preceding Cd stress produced a tolerance effect that prevented membrane damage. Rice seedlings exposed to HS developed subsequent inhibition of Cd-induced ethylene production in detached leaves (Chen and Kao, 1995) and inhibition of Cd-induced oxidative damage of rice seedlings (Hsu and Kao, 2007). HS treatment has also suppressed wound-induced PAL activity in *Lactuca sativa* (Campos-Vargas et al., 2005; Kang and Saltveit, 2003).

Treatment with Cd increases NH_4^+ content and PAL activity in detached rice leaves (Hsu et al., 2006). In rice seedling leaves, $CdCl_2$ increased NH_4^+ content in a sensitive cultivar (Taichung Native 1) but not in a tolerant one (Tainung 67) (Hsu and Kao, 2003). It is not yet known whether HS affects the subsequent Cd-induced increase in NH_4^+ content and PAL activity in leaves of rice seedlings. We thus examined the changes in the content of NH_4^+ and the activity of PAL in rice seedling leaves during Cd stress and then determined whether exposing rice seedling to HS affects the subsequent Cd-induced changes in NH_4^+ content and PAL activity.

MATERIALS AND METHODS

Plant material and growth conditions

Rice (*Oryza sativa* L., cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dishes with wet filter papers at 37°C in the dark. After 48 h incubation, uniformly germinated seeds were selected and cultivated in a beaker containing half-strength Kimura B nutrient solution with the following macro- and micro-elements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4{\cdot}7H_2O$, 91.1 μM KH_2PO_4, 182.5 μM $Ca(NO_3)_2$, 30.6 μM Fe-citrate, 0.25 μM H_3BO_3, 0.2 μM $MnSO_4{\cdot}H_2O$, 0,2 μM $ZnSO_4{\cdot}7H_2O$, 0.05 μM $CuSO_4{\cdot}5\ H_2O$, and 0.07 μM H_2MoO_4. Kimura B solution is considered the most nutritious solution for growing rice plants and is widely used. Since young rice seedlings were used for the present study, the nutrient solution contained no silicon, although silicon is essential for growth of sturdy rice plants in the field. The nutrient solutions (pH 4.7) were replaced every 3 days. The hydroponically cultivated rice seedlings were grown in a Phytotron (Agricultural Experimental Station, National Taiwan University, Taipei, Taiwan) with natural sunlight at 30/25°C day/night and 90% relative humidity.

Cadmium concentration

At the end of treatment, the seedlings were divided into their separate parts (roots and shoots). For Cd determination, shoots and roots were dried at 65°C for 48 h. Dried material was ashed at 550°C for 4 days. The ash residue was incubated with 31% HNO_3 and 17.5% H_2O_2 at 72°C for 2 h, and dissolved in distilled water. Cd concentration was then quantified using an atomic absorption spectrophotometer (Model AA-6800, Shimadzu, Kyoto, Japan). Cd amount was expressed on the basis of dry weight (DW).

HS pretreatment and Cd stress treatment

Twelve-day-old rice seedlings with three leaves were exposed to 30°C (non-HS) and 45°C (HS) for 3 h in the dark. Non-HS and HS seedlings were then grown in half-strength Kimura B solution with or without 5 μM $CdCl_2$ at 30/25°C day/night. Cd-induced chlorosis was first observed visually in the second leaves of rice seedlings. For this reason, the second leaves of rice seedlings were used for the NH_4^+ and PAL analyses.

NH_4^+ determination

The ammonium ion was measured in the crude extract using the Berthelot reaction, modified according to Weatherburn (1967). For NH_4^+ determination in rice seedling leaves, 10 rice leaves were homogenized with a mortar and pestle using 3 mL sulphuric acid (0.3 mM, pH 3. 5). The homogenate was centrifuged for 10 min at 39,000 *g*. Two hundred μL of clear supernatant were diluted by 0.3 mM sulphuric acid to a final volume of 4 mL. For the color reaction, 0.5 mL of solution A (5 g phenol, 25 g nitroprusside dissolved in 100 mL distilled water) and then 0.5 mL of solution B (40 mL 5% sodium hypochlorite and 2.5 g NaOH were mixed and then made up to a final volume of 100 mL with distilled water) were added. Incubation was carried out with gentle shaking in a water bath at 37°C for 20 min. The absorbance was measured at 626 nm against the control without extract. NH_4^+ content was calculated using an extinction coefficient of 3.9982 $\mu mol^{-1}cm^{-1}$ and expressed on the basis of the initial fresh weight (FW).

PAL extraction and assay

Phenyalanine ammonia-lyase was extracted and determined according to Hyodo and Fujinami (1989). The calculation was based on the extinction coefficient (9500 $M^{-1}cm^{-1}$) for *trans*-cinnamic acid. One unit of activity for PAL was defined as the amount of enzyme which caused the formation of 1 μmol *trans*-cinnamic acid per h. The PAL activity was expressed on the basis of mg protein. The enzyme extracts were used for the determination of protein following the method of Bradford (1976).

Statistical analysis

Statistical differences between measurements (n = 4) on different treatments or on different times were analyzed by Duncan's multiple range test. A P<0.05 was considered statistically significant.

RESULTS

Cadmium concentration

To examine the effect of Cd on its concentration in shoots and roots, rice seedlings were grown in nutrient solution with or without 5 μM $CdCl_2$ for 6 days. The Cd concentration in shoots and roots of rice seedlings without Cd was 0.64 ± 0.24 for shoots and 0.78 ± 0.09 μg g^{-1}DW for roots. We observed a marked increase in Cd concentration in Cd-treated shoots and roots (5.89 ± 0.7 for shoots and 11.8 ± 1.7 μg g^{-1}DW for roots).

Changes in NH_4^+ content and PAL activity during Cd stress

To examine the changes in the content of NH_4^+ and the activity of PAL during Cd stress, rice seedlings were grown in nutrient solution with or without 5 μM $CdCl_2$. We observed an increase in the content of NH_4^+ six days after treatment and in the PAL activity caused by $CdCl_2$ four days after treatment (Figure 1A and 1B).

Effects of α-aminooxy-β-phenylpropionic acid

α-Aminooxy-β-phenylpropionic acid (AOPP) is a potent PAL inhibitor (Amrhein and Godeke, 1977). To examine its effect on Cd-increased PAL activity and NH_4^+ content in leaves, rice seedlings were first pretreated with or without 25 μM AOPP for 3 h and then transferred to nutrient solution with or without $CdCl_2$ for six days. We observed that Cd-increased PAL activity and NH_4^+ content in leaves were significantly inhibited by AOPP (Figure 2).

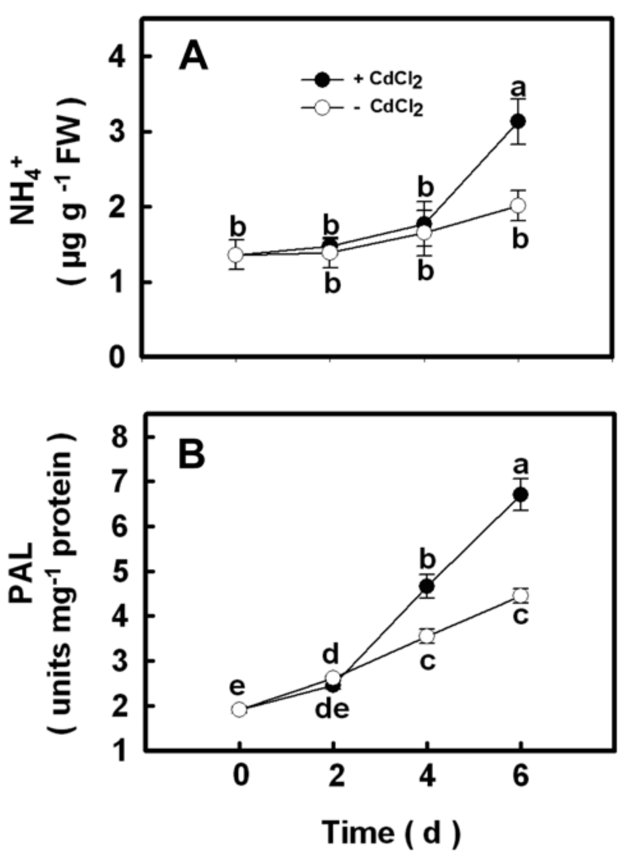

Figure 1. Changes in the content of NH_4^+ (A) and PAL activity (B) in the second leaves of rice seedlings treated with or without 5 μM $CdCl_2$. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

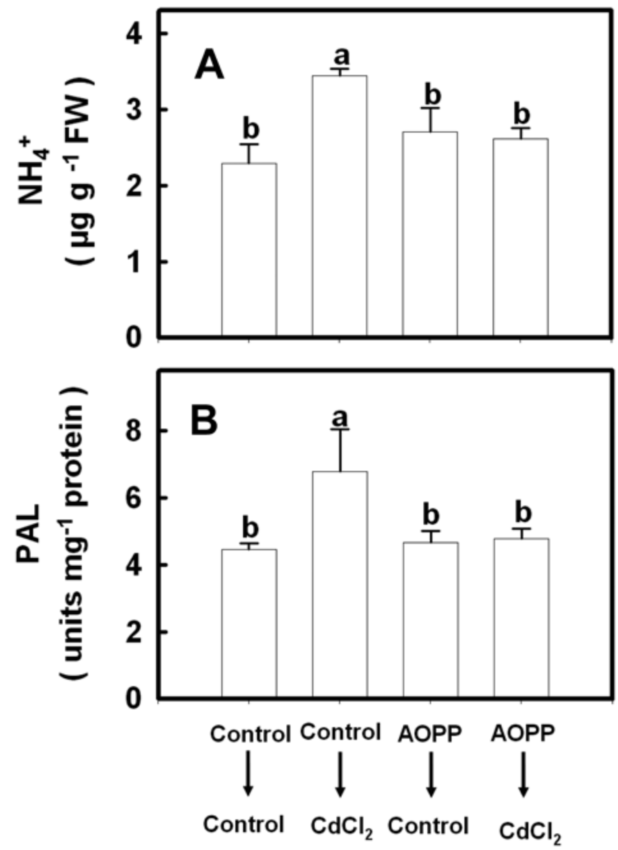

Figure 2. Effect of $CdCl_2$ on the content of NH_4^+ (A) and the activity of PAL (B) in the second leaves of rice seedlings pretreated with or without 25 μM AOPP. Measurements were made 6 d after 5 μM $CdCl_2$ treatment. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

Effects of prior HS exposure

To test whether prior rice seedling exposure to HS affects the subsequent Cd-induced increases in NH_4^+ content and PAL activity in leaves, seedlings were pretreated with HS for 3 h under dark conditions. We observed that a 3 h HS pretreatment reduced both subsequent Cd-induced increases in NH_4^+ content and the PAL activity in rice seedling leaves (Figure 3A and 3B).

Pretreatment with H_2O_2 under non-HS conditions

We reported previously that H_2O_2 content increased in rice seedling leaves after HS exposure (Hsu and Kao, 2007). In view of this result, we studied the effect of H_2O_2 pretreatment under non-HS conditions on Cd-induced NH_4^+ content and PAL activity increases in leaves. To do this, rice seedlings were first pretreated with 0.1 mM H_2O_2 for 3 h under non-HS conditions, then transferred to a nutrient solution with or without $CdCl_2$ for six days. We observed that pretreating rice seedlings with H_2O_2 reduced both the Cd-induced NH_4^+ content and PAL activity increases in leaves (Figure 4A and 4B).

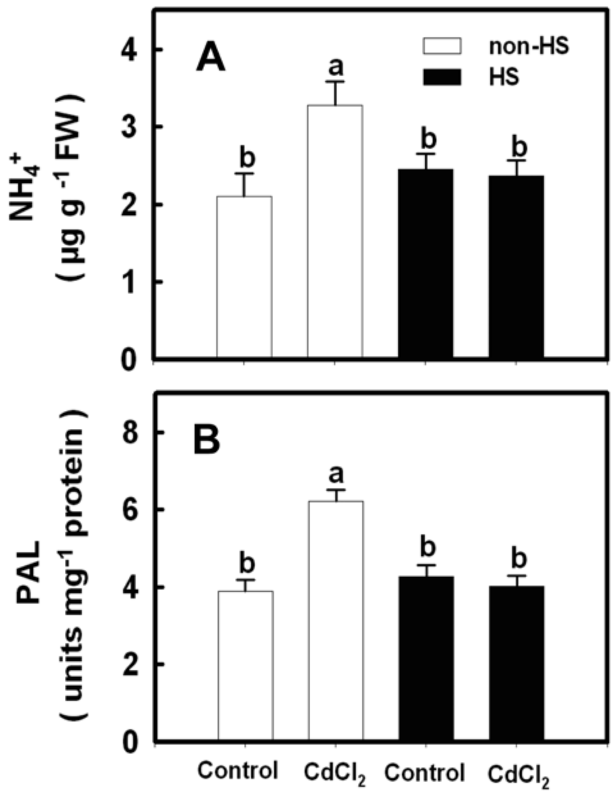

Figure 3. Effect of $CdCl_2$ on the NH_4^+ content (A) and the PAL activity (B) in the second leaves of rice seedlings pretreated with or without HS (45°C) under dark conditions. Measurements were made 6 d after 5 μM $CdCl_2$ treatment. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

Pretreatment with glutathione under non-HS conditions

Glutathione (GSH) is an essential component of the antioxidative defense system, which keeps ROS under control (Noctor and Foyer, 1998). It has been shown that plants under HS conditions have higher GSH content than those that are not (Chao et al., 2009; Nietosotelo and Ho, 1986). Pretreatment of rice seedlings with 1 mM GSH under non-HS conditions for 3 h was effective in reducing both the Cd-induced NH_4^+ content and PAL activity increases in leaves (Figure 4A and 4B).

NADPH oxidase inhibitor effects under HS conditions

We previously demonstrated that HS-increased H_2O_2 production is mediated by a plasma membrane NADPH oxidase in rice seedling leaves (Hsu and Kao, 2007). To confirm the role of H_2O_2 during HS, 0.1 mM imidazole (IMD), a NADPH oxidase inhibitor, was added to the nutrient solution when the third seedling leaves were fully expanded. Figures 5A and 5B show that IMD counteracted both HS-reduced subsequent Cd-induced increases in NH_4^+ content and PAL activity.

Pretreatment with buthionine sulfoximine under HS conditions

Buthionine sulfoximine (BSO) is a GSH biosynthesis inhibitor (Griffith and Meister, 1979). When rice seedlings were pretreated with 0.5 mM BSO for 3 h under HS conditions, the second leaves had a lower GSH content than those pretreated without BSO under the same conditions (Chao et al., 2009). In the present study, we demonstrated that the effect of HS on subsequent Cd-induced increases in NH_4^+ content and PAL activity were diminished by BSO (Figure 5A and 5B). We also observed that the BSO effects under HS conditions were reversed by the application of 1 mM GSH (Figure 5A and 5B).

Pretreatment with lycorine under HS conditions

Rice seedlings under HS conditions have higher ascorbate (AsA) content than those under non-HS conditions (Chao and Kao, 2010). Lycorine (Lyc), an alkaloid extract from members of the Amaryllidaceae, is known to inhibit the conversion of L-galactono-1,4-lactone (GalL) to AsA (Degara et al., 1994). When rice seedlings were pretreated with 0.2 mM Lyc for 3 h under HS conditions, the second leaves had lower AsA contents than those pretreated without Lyc (Chao and Kao, 2010). In the present work, we observed that Lyc reduced the effects of HS on subsequent Cd-induced changes (Figure 5). Moreover, we observed that the Lyc effect under HS conditions was reversed by the addition of 0.5 mM AsA (Figure 5).

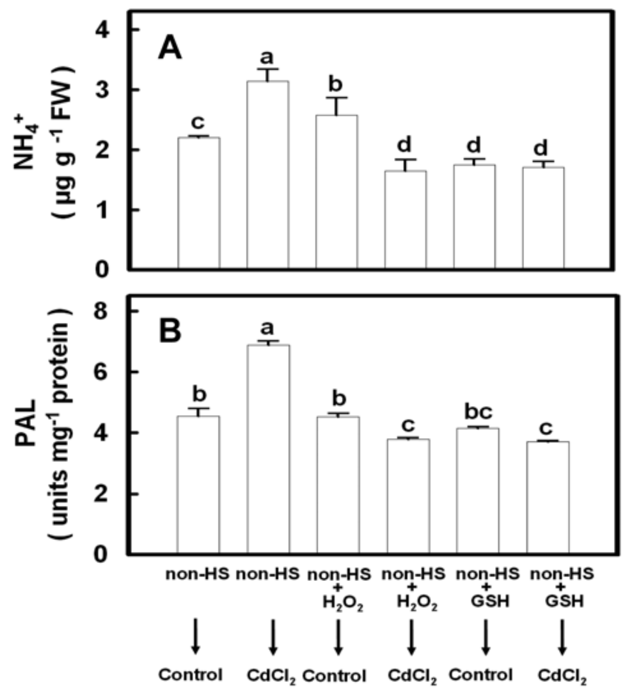

Figure 4. Effect of $CdCl_2$ on the NH_4^+ content (A) and the PAL activity (B) in the second leaves of rice seedlings pretreated with 0.1 mM H_2O_2 or 1 mM GSH under non-HS conditions. Measurements were made 6 d after 5 μM $CdCl_2$ treatment. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

Pretreatment with ascorbate or L-galactono-1, 4-lactone under non-HS conditions

To examine the role of AsA, rice seedlings were pretreated with 0.5 mM AsA or 0.5 mM GalL, a precursor of AsA biosynthesis, under non-HS conditions for 3 h. It was observed that AsA and GalL were effective in reducing subsequent Cd-induced NH_4^+ content and PAL activity in leaves (Figure 6A and 6B).

DISCUSSION

Phenylalanine ammonia-lyase catalyzes the elimination of NH_4^+ from phenylalanine and produces *trans*-cinamate (Griffith and Meister, 1979). We observed that treating rice seedlings with 5 μM $CdCl_2$ resulted in an increase in PAL activity (Figure 1B) and NH_4^+ content (Figure 1A) in leaves. This is consistent with a number of earlier reports (Chien and Kao, 2000; Hsu and Kao, 2003; Hsu and Kao, 2004; Hsu et al., 2006). We also observed that the increase in PAL activity caused by Cd occurs before the NH_4^+ content increase in leaves (Figure 1). AOPP, the hydroxylamine analogue of phenylalanine, is a potent PAL inhibitor (Amrhein and Godeke, 1977). In the present study, we also observed that AOPP inhibited Cd-induced PAL activity and NH_4^+ accumulation in rice seedling leaves (Figure 2A and 2B). It appears that Cd-induced NH_4^+ accumulation in rice seedling leaves is mediated through the increase in PAL activity.

In the previous work, we observed that 0.5 mM $CdCl_2$ increased NH_4^+ content in Cd- sensitive cultivar Taichung Native 1, which was used in the present study, but not in a Cd- tolerant cultivar Tainung 67 (Hsu and Kao, 2003). The results of the present study indicated that $CdCl_2$ at a lower concentration, 5 μM, also induced an increase in NH_4^+ (Figure 1A) .

It has been shown that a brief HS reduces the rise in wound-induced PAL activity in lettuce (Campos-Vargas et al., 2005; Kang and Saltveit, 2003). In rice, we have demonstrated that exposing seedlings to HS results in subsequent inhibition of Cd-induced ethylene production and chlorosis (Chen and Kao, 1995; Hsu and Kao, 2007). Since NH_4^+ accumulation and an increase in PAL activity in rice leaves are Cd-induced effects (Figure 1), exposing seedlings to HS is expected to reduce their subsequent Cd-increased NH_4^+ content and PAL activity. As indicated in Figure 3, this is indeed the case.

The protective effect of HS against subsequent Cd-induced NH_4^+ accumulation and PAL activity in rice seedling leaves is unlikely, due to the inhibition of Cd uptake or transport. This is because rice seedlings pretreated with HS had similar Cd concentrations in leaves caused by $CdCl_2$ as those non-HS (Hsu and Kao, 2007).

Several plants exhibit an early rise in H_2O_2 content during HS (Dat et al., 1998; Gong et al., 2001). In a previous study, we showed that HS pretreatment of rice seedlings results in an increase in H_2O_2 content in 1 h and that HS-

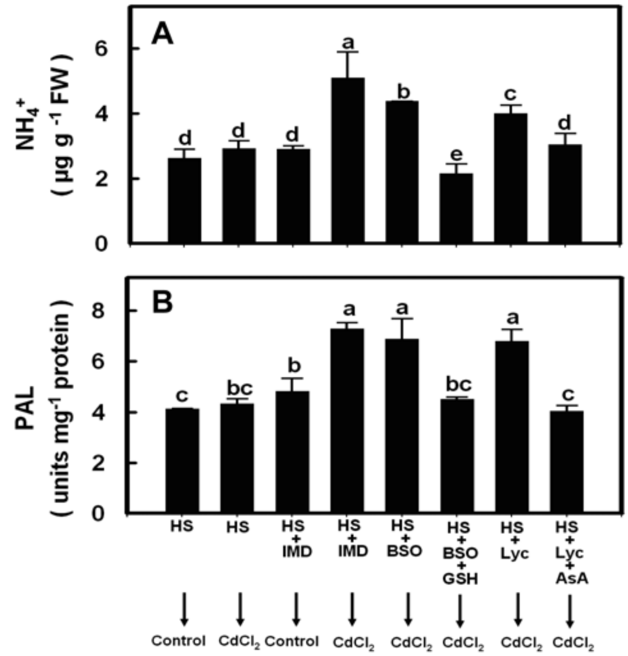

Figure 5. Effect of $CdCl_2$ on the NH_4^+ content (A) and the PAL activity (B) in the second leaves of rice seedlings pretreated with 0.1 mM IMD, 0.5 mM BSO, 1 mM GSH, or 0.2 mM Lyc or 0.5 mM AsA under HS conditions. Measurements were made 6 d after 5 μM $CdCl_2$ treatment. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

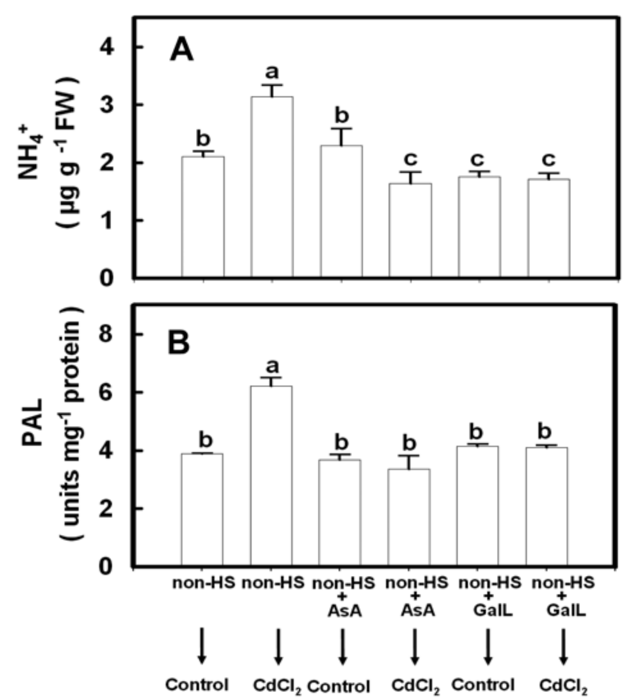

Figure 6. Effect of $CdCl_2$ on the NH_4^+ content (A) and PAL activity (B) in the second leaves of rice seedlings pretreated with 0.5 mM AsA or 0.5 mM GalL under non-HS conditions. Measurements were made 6 d after 5 μM $CdCl_2$ treatment. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

dependent H_2O_2 generation in rice leaves originates in plasma-membrane NADPH oxidase (Hsu and Kao, 2007). In this study, we observed that pretreating rice seedlings with exogenous H_2O_2 under non-HS conditions greatly reduced their Cd-increased NH_4^+ content and PAL activity (Figure 4A and 4B). We also observed that HS-induced protection against subsequent Cd-induced NH_4^+ accumulation and PAL activity can be counteracted by IMD, a NADPH oxidase inhibitor (Figure 5A and 5B). It appears that H_2O_2 is involved in HS-induced protection against subsequent changes in NH_4^+ content and PAL activity caused by Cd.

We recently reported that GSH and AsA are involved in HS-induced Cd tolerance in rice seedlings (Chao and Kao, 2010; Chao et al., 2009). The present study indicates that HS-induced protection against subsequent Cd-induced increases in NH_4^+ content and PAL activity is also mediated by GSH or AsA. This conclusion is supported by our observations that (a) an exogenous supply of GSH, AsA, or GalL under non-HS conditions protected against subsequent Cd-induced decreases in NH_4^+ content and PAL activity in leaves (Figure 4 and 6), (b) pretreatment with BSO under HS conditions reduced GSH content (Chao et al., 2009) and enhanced subsequent Cd effects on NH_4^+ content and PAL activity (Figure 5), (c) the effect of BSO can be reversed by the addition of GSH (Figure 5), (d) pretreatment with Lyc, which is known to inhibit the conversion of GalL to AsA, under HS conditions, reduced AsA content (Chao and Kao, 2010) and enhanced subsequent Cd-increased NH_4^+ content and PAL activity (Figure 5), and (e) the effects of Lyc can be reversed by the addition of AsA (Figure 5).

We have previously shown that paraquat, a well known ROS generating chemical, increases the NH_4^+ content of rice leaves in the light (Chien et al., 2002). Wound-induced PAL activity is now associated with O_2^- production ability in potato tuber (Kumar and Knowles, 2003). It appears that the Cd-induced increases in NH_4^+ content and PAL activity in rice seedlings result from oxidative damage.

It is well established that GSH and AsA are important antioxidant system compounds, that scavenge ROS under oxidative conditions. Increasing evidence indicates that H_2O_2 functions as a signaling molecule in plants. In a previous study, we showed that the accumulation of H_2O_2 precedes GSH or AsA increases during rice seedling HS (Chao and Kao, 2010; Chao et al., 2009). It appears that early accumulation of H_2O_2 during HS signals the increase in GSH or AsA, which in turn protects against subsequent Cd-induced increases in NH_4^+ content and PAL activity in rice seedling leaves (Figure 7). Heat shock proteins (hsps) induced heavy metal tolerance in plants (Neumann et al., 1994). Kang and Saltveit (2003) demonstrated that HS-induced synthesis hsps (e.g. hsp 23) is correlated with the reduction of PAL activity in wounded lettuce tissue. Hsps is thus an alternative mechanism that may explain HS-acquired protection against the increase in NH_4^+ content and PAL activity in rice leaves caused by Cd . Whether the expression of hsps is responsible for HS-induced protection against subsequent Cd-induced increase in NH_4^+ content and PAL activity remains to be established.

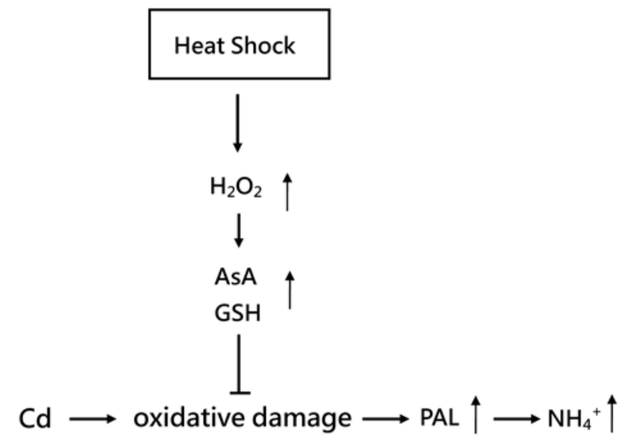

Figure 7. Proposed mechanisms of the HS protection effect against subsequent Cd effect.

By using both a Cd-sensitive cultivar (Taichung Native 1, the cultivar used in the present study) and a Cd-tolerant cultivar (Tainung 67), we showed that NH_4^+ accumulation is involved in regulating the $CdCl_2$-derived toxicity of rice seedlings (Hsu and Kao, 2003). Our present results reconfirm our previous results stating that HS protects against Cd toxicity in rice seedlings (Chao and Kao, 2010; Chao et al., 2009; Hsu and Kao, 2007).

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LITERATURE CITED

Amrhein, N. and K.H. Godeke. 1977. α-Aminooxy-β-phenylpropionic acid - potent inhibitor of L-phenylalanine ammonia-lyase *in vitro* and *in vivo*. Plant Sci. Lett. **8:** 313-317.

Bradford, M.M. 1976. 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.

Campos-Vargas, R., H. Nonogaki, T. Suslow, and M.E. Saltveit. 2005. Heat shock treatments delay the increase in wound-induced phenylalanine ammonia-lyase activity by altering its expression, not its induction in Romaine lettuce (*Lactuca sativa*) tissue. Physiol. Plant. **123:** 82-91.

Chao, Y.Y. and C.H. Kao. 2010. Heat shock-induced ascorbic acid accumulation in leaves increases cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Soil **336:** 39-48.

Chao, Y.Y., Y.T. Hsu, and C.H. Kao. 2009. Involvement of glutathione in heat shock- and hydrogen peroxide-induced cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Soil **318:** 37-45.

Chen, S.L. and C.H. Kao. 1995. Prior temperature exposure affects subsequent Cd-induced ethylene production in rice leaves. Plant Sci. **104:** 135-138.

Chien, H.F. and C.H. Kao. 2000. Accumulation of ammonium in rice leaves in response to excess cadmium. Plant Sci. **156:** 111-115.

Chien, H.F., C.C. Lin, J.W. Wang, C.T. Chen, and C.H. Kao. 2002. Changes in ammonium ion content and glutamine synthetase activity in rice leaves caused by excess cadmium are a consequence of oxidative damage. Plant Growth Regul. **36:** 41-47.

Cho, U.-H. and N.-H. Seo. 2005. Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci. **168:** 113-120.

Dat, J.F., C.H. Foyer, and I.M. Scott. 1998. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. Plant Physiol. **118:** 1455-1461.

Degara, L., C. Paciolla, F. Tommasi, R. Liso, and O. Arrigoni. 1994. *In vivo* inhibition of galactono-γ-lactone conversion to ascorbate by lycorine. J. Plant Physiol. **144:** 649-653.

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

Givan, C.V. 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry **18:** 375-382.

Gong, M., B. Chen, Z.G. Li, and L.H. Guo. 2001. Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2. J. Plant Physiol. **158:** 1125-1130.

Gratao, P.L., A. Polle, P.J. Lea, and R.A. Azevedo. 2005. Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol. **32:** 481-494.

Griffith, O.W. and A. Meister. 1979. Potent and specific-inhibition of glutathione synthesis by buthionine sulfoximine (S-*n*-butyl homocysteine sulfoximine). J. Biol. Chem. **254:** 7558-7560.

Hahlbrock, K. and H. Grisebach. 1979. Enzymic controls in the biosynthesis of lignin and flavonoids. Annu. Rev. Plant Physiol. Plant Mol. Biol. **30:** 105-130.

Hahlbrock, K. and D. Scheel. 1989. Physiology and molecular-biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. **40:** 347-369.

Hsu, Y.T. and C.H. Kao. 2003. Accumulation of ammonium ion in cadmium tolerant and sensitive cultivars of *Oryza sativa*. Plant Growth Regul. **39:** 271-276.

Hsu, Y.T. and C.H. Kao. 2004. Cadmium toxicity is reduced by nitric oxide in rice leaves. Plant Growth Regul. **42:** 227-238.

Hsu, Y.T. and C.H. Kao. 2007. Toxicity in leaves of rice exposed to cadmium is due to hydrogen peroxide accumulation. Plant Soil **298:** 231-241.

Hsu, Y.T., M.C. Kuo, and C.H. Kao. 2006. Cadmium-induced ammonium ion accumulation of rice seedlings at high temperature is mediated through abscisic acid. Plant Soil **287:** 267-277.

Hyodo, H. and H. Fujinami. 1989. The effects of 2,5-norbornadiene on the induction of the activity of 1-aminocyclopropane-1-carboxylate synthase and of phenylalanine ammonia-lyase in wounded mesocarp tissue of *Cucurbita Maxima*. Plant Cell Physiol. **30:** 857-860.

Kang, H.M. and M.E. Saltveit. 2003. Wound-induced PAL activity is suppressed by heat-shock treatments that induce the synthesis of heat-shock proteins. Physiol. Plant. **119:** 450-455.

Kumar, G.X.M. and N.R. Knowles. 2003. Wound-induced superoxide production and PAL activity decline with potato tuber age and wound healing ability. Physiol. Plant. **117:** 108-117.

Miflin, B.J. and P.J. Lea. 1976. Pathway of nitrogen assimilation in plants. Phytochemistry **15:** 873-885.

Neumann, D., O. Lichtenberger, D. Gunther, K. Tschiersch, and L. Nover. 1994. Heat-shock proteins induce heavy-metal tolerance in higher plants. Planta **194:** 360-367.

Nietosotelo, J. and T.H.D. Ho. 1986. Effect of heat-shock on the metabolism of glutathione in Maize roots. Plant Physiol. **82:** 1031-1035.

Noctor, G. and C.H. Foyer. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49:** 249-279.

Orzech, K.A. and J.J. Burke. 1988. Heat-shock and the protection against netal toxicity in wheat leaves. Plant Cell Environ. **11:** 711-714.

Sanitá di Toppi, L. and R. Gabbrielli. 1999. Response to cadmium in higher plants. Environ. Exp. Bot. **41:** 105-130.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. **39:** 971-974.

水稻幼苗經熱休克前處理可降低鎘所誘導葉片中銨離子之累積與苯丙胺酸氨裂解酶活性之增加

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本研究主要探討水稻幼苗經熱休克處理後，對鎘所誘導葉片中銨離子累積與增加苯丙胺酸氨裂解酶 (PAL) 活性之影響。鎘所誘導葉片中 PAL 活性增加之時間早於銨離子累積。鎘所誘導 PAL 活性與銨離子含量之增加會因 PAL 抑制劑 α-aminooxy-β-phenylpropionic acid 處理而降低。另外，水稻幼苗在黑暗中經熱休克前處理 3 小時後，可抑制後續鎘所誘導 PAL 活性增加與銨離子累積。在不經熱休克前處理情況下，外加過氧化氫，穀胱甘肽（GSH）、抗壞血酸 (AsA) 與抗壞血酸之前驅物（L-galactono -1,4-lactone）所顯示之效果與處理熱休克結果相似。我們亦發現水稻幼苗處理 imidazole（NADPH oxidase 抑制劑）、buthionine sulfoximine（BSO，穀胱甘肽合成抑制劑）及 lycorine（Lyc, 抗壞血酸合成抑制劑）等抑制劑時，可抵銷熱休克處理所減緩鎘誘導之 PAL 活性增加與銨離子累積。此外，分別外加 GSH 與 AsA 亦可恢復被 BSO 與 Lyc 所抑制的效果。本文亦對水稻經熱休克處理後減緩鎘作用可能的機制加以討論。

關鍵詞：銨離子；鎘；熱休克；水稻；氧化逆境；苯丙胺酸氨裂解酶。