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Abscisic acid is an inducer of hydrogen peroxide production in leaves of rice seedlings grown under potassium deficiency

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ABSTRACT. Potassium (K) is essential for many physiological processes. K deficiency is known to increase the production of reactive oxygen species including H_2O_2 in bean and Arabidopsis. Accumulating evidence indicates that the action of abscisic acid (ABA) is associated with H_2O_2 production. In this study, we examined the possible involvement of ABA in K deficiency-induced H_2O_2 production in the leaves of rice seedlings. The histochemical and colorimetrical methods were used to determine H_2O_2 production in the leaves of rice seedlings. It was observed that K deficiency resulted in an increase in H_2O_2 content in the leaves. H_2O_2 production in rice leaves induced by K deficiency was blocked by diphenyleneiodonium chloride and imidazole, NADPH oxidase inhibitors. In this study, ABA content was judged by the expression of *OsRab16A* (an ABA responsive gene) or was determined by the enzyme-linked immunosorbent assay. K deficiency also resulted in an increase in ABA content in rice leaves. However, ABA accumulation in the leaves under K deficiency is not induced by H_2O_2. Tungstate (Tu), an ABA biosynthesis inhibitor, was effective in reducing K deficiency-increased ABA content, as well as K deficiency-induced H_2O_2 production, indicating that K deficiency-induced H_2O_2 in rice leaves is mediated through ABA. This conclusion is supported further by the observation that exogenous ABA treatment increased H_2O_2 content in the leaves of rice seedlings grown under K-sufficient conditions.

Keywords: Abscisic acid; Hydrogen peroxide; Potassium deficiency; *Oryza sativa* L.

INTRODUCTION

Crop plants have a fundamental dependence on the use of potash fertilizer. The lack of potassium (K) is a key limiting factor of crop yields on agricultural soils (Pettigrew, 2008). As K fertilizer becomes more expensive and as farmers reduce usage of K fertilizer because of the negative environmental impacts, it will be important to gain a better understanding of how crop plants response to lower K input.

Potassium is the most abundant inorganic cation in plants (Leigh and Wyn Jones, 1984). K accumulates to a considerable concentrations in cytosolic and vacuolar compartments. Thus, its role in plant physiology can roughly be distributed between cytoplasm and vacuole. K plays a central role in maintenance of photosynthesis. It has been shown that the rate of net photosynthesis and the activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) decrease in plants under conditions of K deficiency (Peoples and Koch 1979; Zhao et al. 2001; Cakmak 2005; Weng et al., 2007). To maintain photosynthesis at high rate, export of sucrose within plants is required. There are reports showing that K deficiency causes a decrease in sucrose export from source leaves (Mengel and Viro, 1974; Cakmak, 2005). The impairment in photosynthetic CO_2 fixation and decrease in sucrose export in K-deficient leaves cause an over-reduction in the photosynthetic electron transport chain that potentiates the generation of reactive oxygen species (ROS) (Cakmak, 2005).

ROS originating from the plasma-membrane NADPH oxidase, which transfers electrons from cytoplasmic NADPH to O_2 to form $O_2^{\cdot-}$, followed by dismutation to H_2O_2, has been a recent focus in ROS signaling research. Beside the photosynthetic electron transport, NADPH oxidase represents another source for production of ROS in plant cells. Cakmak (2005) demonstrated that the activity of NADPH oxidase and NADPH-dependent $O_2^{\cdot-}$ generation indeed increase in K-deficient bean roots.

The plant hormone abscisic acid (ABA) is known to regulate plant adaptive responses to various adverse environmental conditions (Wasilewska et al., 2008). It is well established that ABA accumulates in plants under drought conditions. It has been shown that ABA content accumulates in the mineral-deprived plants (Mizrahi and Rich-

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mond, 1972; Vysotskaya et al., 2008). Only few data have been published on the effect of K deficiency on ABA relations in plants. A decreased ABA accumulation has been observed in embryos of sorghum (Benech et al., 1995). However, slightly increased ABA content in grains and an enhanced ABA content in flag leaves from K-deficient wheat plants have been observed (Haeder and Beringer, 1981). There are other reports showing that ABA was found to accumulate in roots rather than shoot under K deficiency (Peuke et al., 2002; Schraut et al., 2005). Thus, K deficiency has inconsistent effect on ABA.

Accumulating evidence indicates that one mechanism of ABA action is associated with ROS production in plant cells. It has been shown that ABA can increase the generation of H_2O_2 and O_2^- (Guan et al., 2000; Jiang and Zhang, 2001; Lin and Kao, 2001; Hung and Kao, 2004; Tsai et al., 2004; Hu et al., 2006) in plant tissues. Because H_2O_2 is a relatively stable and diffusible through membrane, H_2O_2 is thought to constitute a general signaling molecule (Quan et al., 2008). Water stress-induced ABA accumulation has been shown to trigger the increased generation of H_2O_2 in maize leaves (Jiang and Zhang, 2002a, b; Hu et al., 2006). However, it is not known whether K deficiency-induced ABA accumulation affects H_2O_2 production in plants.

Little is known about the responses of rice seedlings to potassium deficiency. In this study, we described the effect of K deficiency on the contents of ABA and H_2O_2 in leaves of rice seedlings. The objective of the present study was to find out the contribution of endogenous ABA to K deficiency-induced H_2O_2 production in leaves of rice seedlings under conditions of K deficiency.

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 wetted 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 sufficient K supply (control) or deficient K supply (-K). Nutrient solution for the control contains the following macro- and micro-elements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4 \cdot 7\ H_2O$, 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 $ZnSO_4 \cdot 7\ H_2O$, 0.05 μM $CuSO_4 \cdot 5H_2O$ and 0.07 μM H_2MoO_4 (Kimura, 1931). Sodium nitrate substituted for KNO_3 and NaH_2PO_4 for KH_2PO_4, respectively, under K-deficient conditions. Kimura B nutrient solution contains the desired nutrients for growing rice plants. 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 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. When the third leaves of control or -K seedlings were fully grown, the second leaves of control and -K seedlings were excised to determine the contents of ABA and H_2O_2. The time of different treatments (including K deficiency, H_2O_2, ABA, ABA inhibitor, and NADPH inhibitors) used in this study was basically according to our preliminary experiments.

Determination of K

For determination of K, samples were dried at 65°C for 2 days. Dried material was ashed at 550°C for 4 days. The ash residue was incubated with 70% HNO_3 and 30% H_2O_2 at 72°C for 2 h, and dissolved in distilled water. K concentrations were then quantified using an atomic absorption spectrophotometer (Model AA-6800, Shimadzu, Kyoto, Japan) and expressed on the basis of DW.

Growth response

At the end of treatment, the seedlings were divided into shoots and roots. For DW estimation, the shoots and roots were dried at 65°C for 48 h.

Determination of H_2O_2

To visualize H_2O_2 *in situ*, 3,3-diaminobenzidine (DAB) staining was performed as described previously (Hsu and Kao, 2007). The H_2O_2 content was also measured colorimetrically. H_2O_2 was extracted with sodium phosphate buffer (50 mM, pH 6.8) containing 1 mM hydroxylamine, a catalase inhibitor. The H_2O_2 content was measured after reaction $TiCl_4$ (Tsai et al., 2004). The blank reaction consisted of 50 mM phosphate buffer in the absence of leaf extracts. The absorbance was measured at 410 nm. The amount of H_2O_2 was calculated by using a standard curve prepared with known concentrations of H_2O_2.

Determination of ABA

For extraction of ABA, leaves were homogenized with a pestle and mortar in extraction solution (80% methanol containing 2% glacial acetic acid). To remove plant pigments and other non-polar compounds which could interfere in the immunoassay extracts were first passed through a polyvinylpyrrolidone column and C18 (Sep-Pak Vac) cartridges (Waters, Milford, MA, USA). The eluates were concentrated to dryness by vacuum evaporation and resuspended in Tris-buffered saline before enzyme-linked immunosorbent assay (ELISA). ABA was quantified by ELISA. The ABA immunoassay detection kit (Phytodek) was purchased from Agdia (Elkhart, IN, USA) and is specific for (+)-ABA. By evaluating [^{3}H]ABA recovery, [^{3}H] ABA loss was <3% by the method described here. The content of ABA was expressed on the basis of fresh weight (FW).

The *Rab16A* is an ABA responsive rice gene (Mundy and Chua, 1988; Hong et al., 2009). Thus, in some experi-

Table 1. Primers used in semi-quantitative RT-PCR assay.

Gene	TIGR locus name	Primer	Sequence (5′ to 3′)	Products (bp)
OsRab16A	LOC_Os11g26790.1	*Rab16A-5′*	CACAGTACAAACAACACGCAGACA	105
		Rab16A-3′	CCGAGCGCAATAAAAGGAAA	
OsUbiquitin	LOC_Os03g13170.1	*Ubi-5′*	CGCAAGTACAACCAGGACAA	101
		Ubi-3′	TGGTTGCTGTGACCACACTT	

ments the ABA level was also judged by the expression of *OsRab16A*. Semi-quantitative RT-PCR analysis was used to examine the expression of *OsRab16A*. Total RNA was isolated from the second leaves of seedlings with use of TRIzol reagent (Invitrogen, CA, USA), according to supplier's recommendations. To prevent DNA contamination, RNA was treated with Turbo DNase I (Ambion, TX, USA) for 30 min at 37°C before RT-PCR analysis. Moreover, the control PCR amplifications involved use of RNA as a template after the DNase I treatment to verify the complete elimination of contaminated DNA. The reverse-transcription reactions involved 200 ng of total RNA by use of the SuperScript III first-strand synthesis RT-PCR system (Invitrogen, CA, USA) according to the manufacturer's protocol.

The gene-specific primer was designed from the 3'UTR of the rice *Rab16A* gene (Hong et al., 2009). The sequences used and the predicted amplicon are listed in Table 1. The RT-PCR program initially started with 94°C denaturation for 5 min, followed by 27 to 29 cycles of 94°C for 30 s, 60°C for 70s, 72°C 30 s, 72°C extension for 5 min, and finally keep in 16°C. The PCRs were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. All tests were repeated at least three times, and one of the repeats is shown in the figures. For all treatments, three replicates of RT-PCR were conducted with three batches of total RNA samples isolated independently. PCR products were resolved by elecrophoresis in 3% agarose gel and stained with ethidium bromide. The gel images were digitally captured with use of a SynGene gel documentation system and analyzed with use of Genetools (Syngene, MD, USA). The rice *OsUniquitin* gene was used for normalization.

Statistical analysis

Statistical differences between measurements (n=3 or 4) for different treatments were analyzed following Student's t-test or Duncan's multiple range test. A $P<0.05$ was considered statistically significant.

RESULTS

The effect of K deficiency on growth response and K concentration

To study the effect of K deficiency on growth response and K accumulation, rice seedlings were grown under K-sufficient (control) and -deficient conditions for 12 days. The FW and DW of both shoots and roots were not affected by K deficiency (Table 2). However, K deficiency resulted in a decrease in K concentration in shoots and roots (Table 2).

H_2O_2 accumulation is induced by K deficiency

In the present study, H_2O_2 production was first visualized by a histochemical method with DAB that is based on the formation by H_2O_2 of brown polymerization product. The DAB staining method for H_2O_2 in the second leaves of rice seedlings is specific, because the development of DAB-H_2O_2 reaction product could be prevented by ascorbic acid, a H_2O_2 scavenger (Hsu and Kao, 2007). Here, we observed that K deficiency led to an accumulation of DAB-H_2O_2 reaction product (Figure 1A). When H_2O_2 was measured colorimetrically, K deficiency also resulted in an increase in H_2O_2 content in the second leaves (Figure 1B).

The effect of NADPH oxidase inhibitors on H_2O_2 production

The role of NADPH oxidase in K deficiency-stimulated H_2O_2 production was investigated by NADPH oxidese inhibitors such as imidazole (IMD) and diphenyleneiodonium chloride (DPI). When 100 μM IMD or 50 μM DPI was added to the K-deficient nutrition solution for 3 h, a reduction of H_2O_2 accumulation induced by K deficiency was observed (Figures 2A and 2B).

ABA accumulation is induced by K deficiency

Rab16A (initially called *Rab21*) mRNA is known to increase in rice embryos, leaves, roots, and callus derived

Table 2. The FW, DW, and K concentration in shoots and roots of rice seedlings growth under K-sufficient (control) and -deficient (-K) condition for 12 days (n=4).

Treatment	FW (mg seedling^{-1})		DW (mg seedling^{-1})		K (mg g^{-1} DW)	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	51.9 ± 1.6	31.5 ± 1.9	9.2 ± 0.3	4.9 ± 0.2	1.5 ± 0.2	5.2 ± 0.8
-K	52.6 ± 2.4	32.3 ± 2.3	8.8 ± 0.2	4.5 ± 0.4	0.6 ± 0.3*	0.6 ± 0.03*

Asterisks represent values that significantly different between control and -K treatments at $P<0.05$.

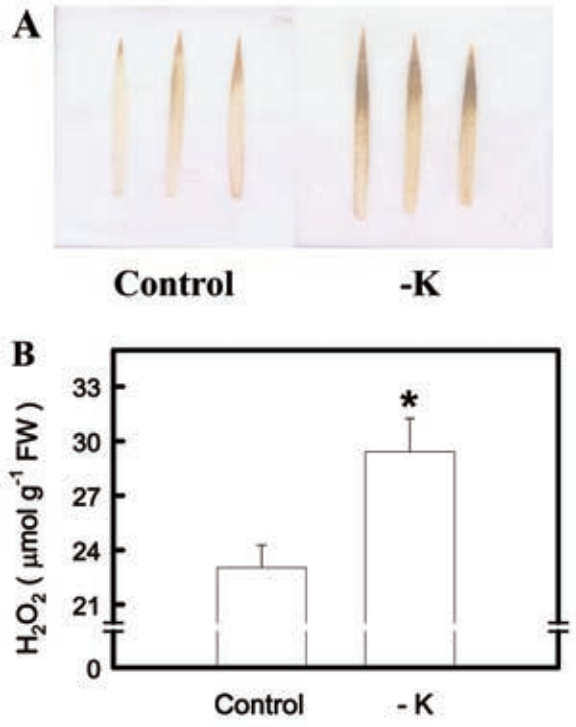

Figure 1. DAB-H_2O_2 reaction product (A) and H_2O_2 content (B) in the second leaves of rice seedlings grown under K-sufficient (control) and -deficient (-K) conditions for 12 days. Bars show means ± SE (n = 4). Asterisk represents values that are significantly different between control and -K treatments at P<0.05.

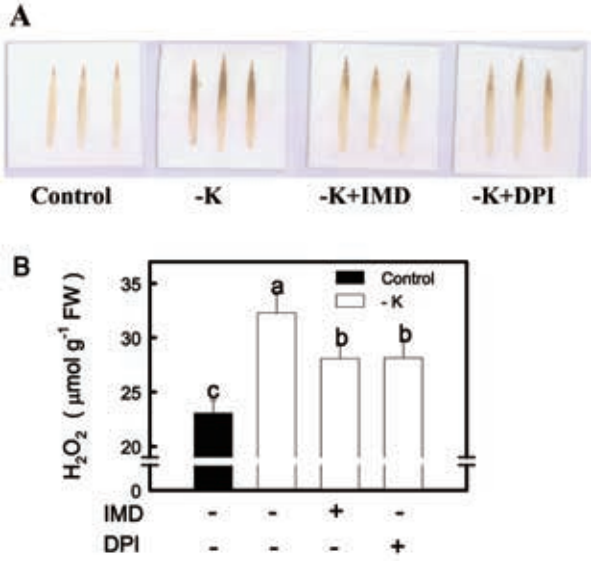

Figure 2. Effect of IMD and DPI on DAB-H_2O_2 reaction product (A) and H_2O_2 content (B) in the second leaves of rice seedlings grown under K-deficient (-K) conditions. Rice seedlings were first grown under -K conditions for 12 days then transferred to -K nutrient solution with or without 100 μM IMD and 50 μM DPI for another 3 h. Bars show means ± SE (n = 4). Values with the same letter are not significantly different at P<0.05.

suspension cells on treatment with ABA (Mundy and Chua, 1988; Hong et al., 2009). Thus, ABA content in this study was judged by the transcripts of *OsRab16A*. Figure 3A shows that the expression of *OsRab16A* was increased in K-deficient leaves. When ABA content was determined by the ELISA, we also found that K deficiency resulted in an increase in ABA content in the second leaves (Figure 3B).

ABA accumulation is not induced by H_2O_2

To examine if ABA accumulation induced by K defi-

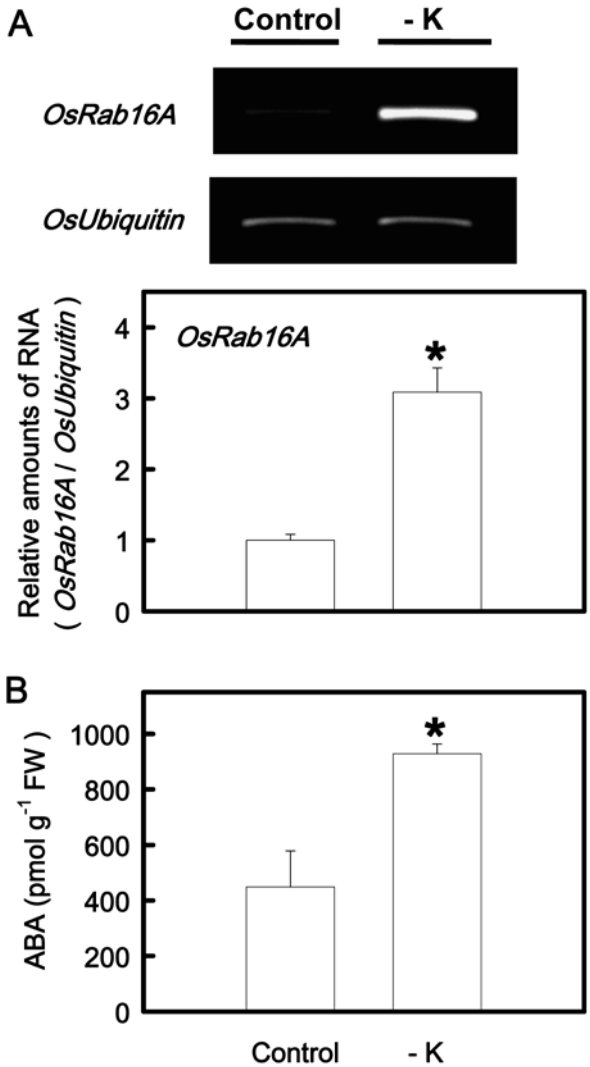

Figure 3. The mRNA level of *OsRab16A* (A) and the content of ABA (B) in the second leaves of rice seedlings grown under K-sufficient (control) and -deficient (-K) conditions for 12 days. The value of *OsRab16A* gene was adjusted by a corresponding amount of *OsUbiquitin*. After the adjustment by *OsUbiquitin*, the reaction of the control was treated as the normalized reference, with a value of one, for determining the relative amount of mRNA of *OsRab16A* gene. Bars show means ± SE (n = 3 for *OsRab16A* transcripts and n = 4 for ABA content). Asterisks represent values that are significantly different between control and -K treatments at P<0.05.

ciency is mediated through H_2O_2, rice seedlings were first grown in K-deficient conditions for 12 days, by which time the third leaves were fully grown. Rice seedlings were then grown in K-deficient nutrient solution with or without 100 μM IMD for 3 h. We observed that the transcript of *OsRab16A* in K-deficient leaves was not reduced by IMD treatment (Figure 4).

To test if H_2O_2 plays an important role in regulating ABA accumulation, 12-day-old control (K-sufficient) seedlings were grown in K-sufficient nutrient solution with or without 0.5 mM H_2O_2 for 6 h. The second leaves of rice seedlings were then used to determine the expression of *OsRab16A*. Application of H_2O_2 increased H_2O_2 content in the second leaves of K-sufficient rice seedlings (Figure 5A). However, the expression of *OsRab16A* was not affected by H_2O_2 (Figure 5B).

The effect of sodium tungstate on H_2O_2 production under K-deficient conditions

Sodium tungstate (Tu) is known to block the formation of ABA from abscisic aldehyde by impairing abscisic aldehyde oxidase (Hansen and Grossmann, 2000). When 12-day-old K-deficient seedlings were grown in K-deficient nutrient solution in the presence or absence of 1 mM Tu for 1 day, the ABA content increased by K deficiency was significantly reduced by Tu (Figure 6A). Moreover, Tu was effective in reducing H_2O_2 production induced by K deficiency (Figures 6B and 6C).

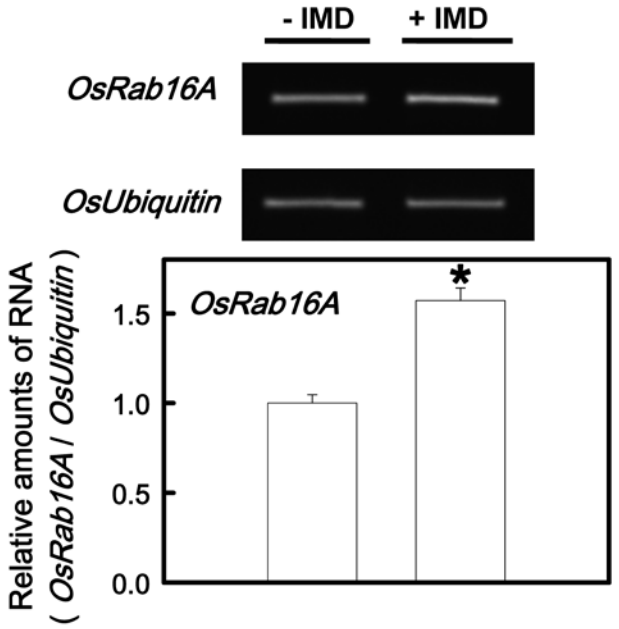

Figure 4. Effect of IMD on the mRNA level for *OsRab16A* in the second leaves of rice seedlings grown under K-deficient (-K) conditions. Rice seedlings were first grown under -K conditions for 12 days and then transferred to -K nutrient solution with or without 100 μM IMD for another 3 h. The value of *OsRab16A* gene was adjusted by a corresponding amount of *OsUbiquitin*. After the adjustment by *OsUbiquitin*, the reaction of -IMD was treated as the normalized reference, with a value of one, for determining the relative amount of RNA of *OsRab16A* gene. Bars show means ± SE (n = 3). Asterisk represents values that are significantly different between -IMD and +IMD treatments at P<0.05.

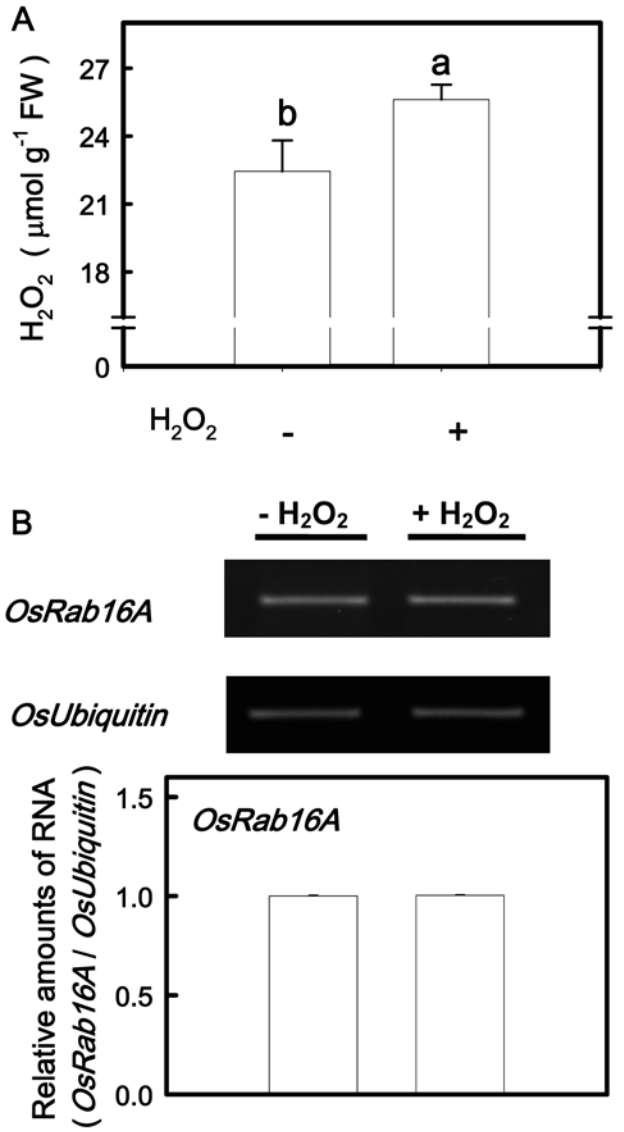

Figure 5. Effect of exogenous H_2O_2 on the content of H_2O_2 (A) and the mRNA level for *OsRab16A* (B) in the second leaves of rice seedlings grown under K-sufficient conditions. Rice seedlings were first grown under K-sufficient conditions for 12 days and then transferred to K-sufficient nutrient solution with or without 0.5 mM H_2O_2 for another 6 h. A: Bars show means ± SE (n = 4). Asterisk represents values that are significantly different between -H_2O_2 and +H_2O_2 treatments at P<0.05; B: The value of *OsRab16A* gene was adjusted by a corresponding amount of *OsUbiquitin*. After the adjustment by *OsUbiquitin*, the reaction of -H_2O_2 was treated as the normalized reference, with a value of one, for determining the relative amount of RNA of *OsRab16A* gene. Bars show means ± SE n= 3. No significant difference in the mRNA level for *OsRab16A* was observed between -H_2O_2 and +H_2O_2 treatments at P<0.05.

ABA increases H_2O_2 production under K-sufficient conditions

To test if ABA regulates H_2O_2 production, 12-day-old K-sufficient seedlings were transferred to K-sufficient nutrient solution with or without 10 μM ABA for 2 days. We observed that ABA treatment resulted in a significant increase in mRNA level of *OsRab16A* (Figure 7A) and H_2O_2 production (Figures 7B and 7C) in the second leaves.

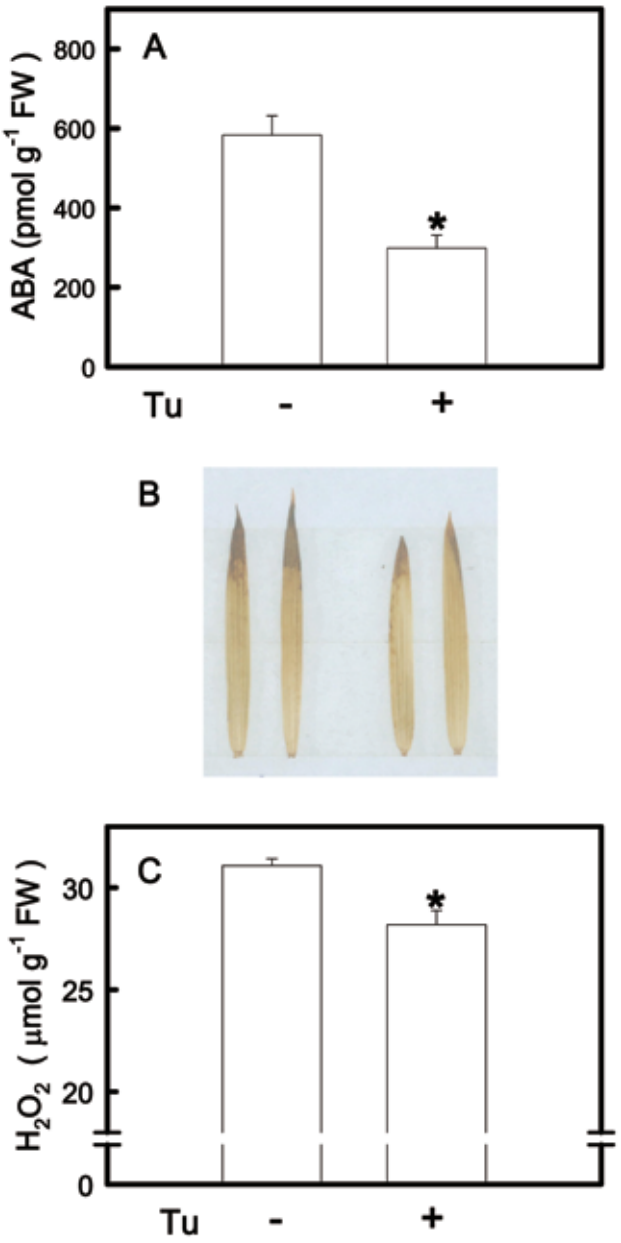

Figure 6. Effect of Tu on the contents of ABA (A), DAB-H_2O_2 reaction product (B), and H_2O_2 content (C) in the second leaves of rice seedlings grown under K-deficient (-K) conditions. Rice seedlings were first grown under -K conditions for 12 days and then transferred to -K nutrient solution with or without 1mM Tu for another 24 h. Bars show means ± SE (n = 4). Asterisks represent values that are significantly different between -Tu and +Tu treatments at P<0.05.

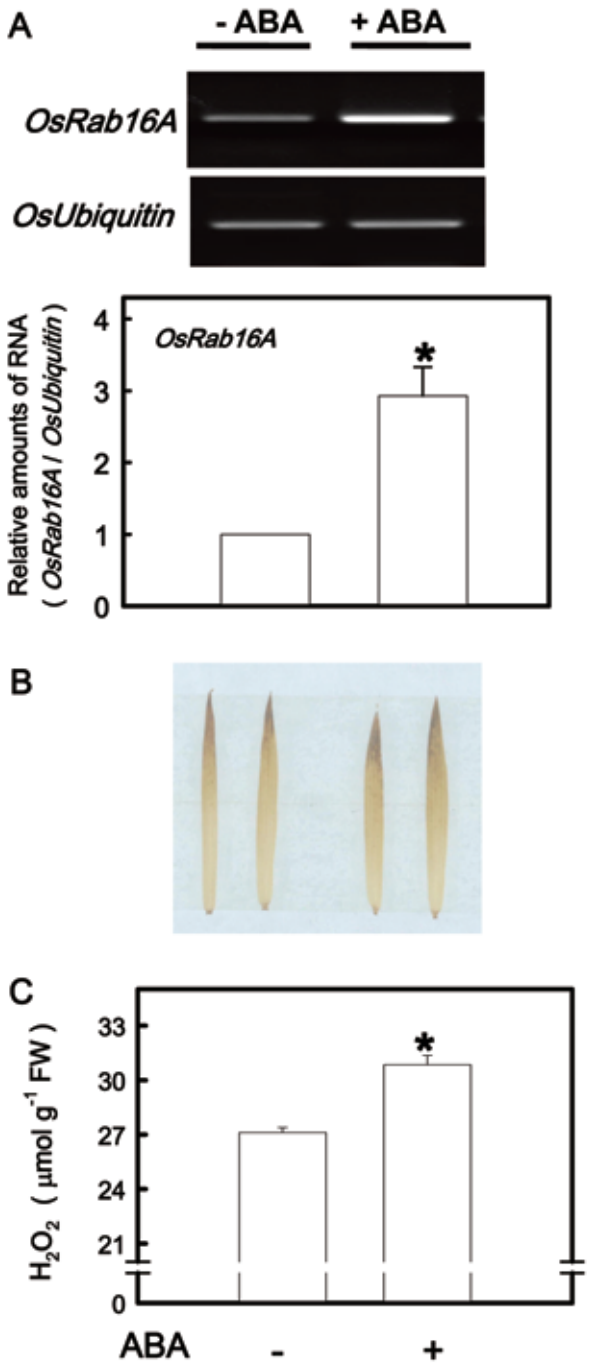

Figure 7. Effect of exogenous ABA on the mRNA level for *OsRab16A* (A), DAB-H_2O_2 reaction product (B) and H_2O_2 content (C) in the second leaves of rice seedlings grown under K-sufficient conditions. Rice seedlings were first grown under K-sufficient conditions for 12 days and then transferred to K sufficient nutrient solution with or without 10 μM ABA for another 2 days. A: The value of *OsRab16A* gene was adjusted by a corresponding amount of *OsUbiquitin*. After the adjustment by *OsUbiquitin*, the reaction of -ABA was treated as the normalized reference, with a value of one, for determining the relative amount of RNA of *OsRab16A* gene. *Bars* show means ± SE (n = 3); B: Bars show means ± SE (n = 4). Asterisk represents values that are significantly different between -ABA and +ABA treatments at P<0.05.

DISCUSSION

In the present study, the histochemical and colorimetrical methods were used to determine H_2O_2 production. These methods have been widely used for the detection of H_2O_2 in plant tissues in response to biotic and abiotic stresses. Our results showed that K deficiency, which decreased K concentration in shoots and roots (Table 2), led to H_2O_2 accumulation in the second leaves of rice seedlings (Figures 1A and 1B). The accumulation of H_2O_2 caused by K deficiency has also been described in bean and Arabidopsis roots (Shin and Schachtman, 2004; Cakmak, 2005; Shin et al., 2005). The application of NADPH oxidase inhibitors IMD and DPI effectively reduced H_2O_2 production in leaves of rice seedlings grown under K deficiency (Figures 2A and 2B). It has been shown that a high concentration of DPI can affect other enzymes potentially involved in the production of ROS, including cell peroxidase and nitric oxide synthase (Bolwell et al., 1998; Orozco-Cárdenas et al., 2001). The fact that K deficiency-induced H_2O_2 accumulation in the second leaves can be inhibited by low concentration (50 μM) DPI, and can be inhibited by both DPI and IMD strongly suggests that K deficiency-dependent H_2O_2 production originated, at least in part from plasma membrane NADPH oxidase. Our results support the findings of Cakmak (2005), who demonstrated that NADPH oxidase activity and NADPH-dependent O_2^- production increase in K-deficient bean roots.

It has been shown that ABA content of leaves increases in the mineral-deprived plants (Mizrahi and Richmond, 1972; Vysotskaya et al., 2008). Haeder and Beringer (1981) demonstrated that ABA content in flag leaves of wheat plants increases under conditions of K deficiency. We also observed that ABA accumulated in K-deficient rice leaves (Figures 3A and 3B). O'Toole and Cruz (1980) reported the linear relationship between leaf rolling and leaf water potential and concluded that leaf rolling in rice could be used as an estimate of water deficit. In the hydroponically grown K-deficient rice seedlings of the present study, no leaf rolling was visually observed (data not shown). Thus, ABA accumulated in rice leaves is directly due to K deficiency.

The fact that application of IMD, which decreased H_2O_2 production (Figure 2A and 2B), was unable to reduce ABA accumulation in K-deficient rice leaves (Figure 4), suggests that K deficiency-dependent ABA accumulation is not due to H_2O_2 accumulation. This suggestion is supported further by the observations that treatment with exogenous H_2O_2, which increased H_2O_2 content (Figure 5A), had no effect on ABA content in K-sufficient rice leaves (Figure 5B).

ABA-induced H_2O_2 production was first observed in guard cells (Pei et al., 2000: Zhang et al., 2001). In subsequent work, ABA-induced increase in H_2O_2 production has been reported for maize seedlings(Jiang and Zhang, 2004; Hu et al., 2006), rice leaves (Hung and Kao, 2004), and rice roots (Hung and Kao, 2004; Tsai et al., 2004). In the present study, we also found that treatment with exogenous ABA, which increased expression of *OsRab16A* (Figure 7A), effectively increased H_2O_2 production in leaves of rice seedling grown under K-sufficient conditions (Figures 7B and 7 C). Moreover, NADPH oxidase has been observed to be the enzyme responsible for H_2O_2 production in ABA-treated rice leaves and roots (Hung and Kao, 2004; Tsai et al., 2004).

Potassium deficiency induces ABA accumulation, and ABA can induce H_2O_2 production in rice leaves. However, it is not known what is the relative contribution of K deficiency-induced ABA accumulation to K deficiency-induced H_2O_2 production. In this study, using an ABA biosynthesis inhibitor Tu, the role of endogenous ABA in the production of H_2O_2 induced by K deficiency was examined. Our results showed that Tu treatment was effective in reducing K deficiency-induced ABA content (Figure 6A), as well as K deficiency-induced H_2O_2 production (Figures 6B and 6C). ABA is proved to be an inducer of H_2O_2 production in rice seedlings under conditions of K deficiency. ABA has also been shown to be effective in inducing H_2O_2 production in guard cells in *Vicia faba* (Zhang et al., 2001) and in the leaves of maize seedling exposed to water stress (Jiang amd Zhang, 2002b).

Sodium tungstate, a molybdate analogue, has also been demonstrated to inhibit the formation of nitric oxide (NO) through nitrate reductase (Bright et al., 2006). Addition of the NO donor sodium nitroprusside to rice seedlings grown under K-sufficient conditions and the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide to rice seedlings grown under K-deficient conditions had no effect on H_2O_2 production (data not shown). Thus, the effect of Tu on H_2O_2 production reported here is mainly due to the inhibition of ABA biosynthesis rather than the inhibition of NO production.

Results observed in the present study suggest that NADPH oxidase, which shows sensitivity to DPI and IMD, is operating in K-deficient rice leaves. It appears that when rice seedlings are grown under K deficiency conditions, H_2O_2 in rice leaves generated in the apoplast. Water channels (aquporins) may serve as conduits for transmembrane H_2O_2 transport (Henzler and Steudle, 2000). In general, the apoplast represents only a small proportion of the cell's antioxidant capacity. H_2O_2 in the apoplast likely rapidly moves into the cytosol to function as a mobile signaling molecule, but whether H_2O_2 is the sole signal in K-deficient rice leaves remains to be determined.

In conclusion, the data obtained in this study clearly suggests that K deficiency-induced ABA accumulation triggers the increased production of H_2O_2, which may involve a plasma-membrane NADPH oxidase, in the leaves of rice seedlings. To our knowledge, this is the first report indicating that ABA is an inducer of H_2O_2 production in plants under condition of K deprivation

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水稻葉苗葉片在缺鉀狀況下所形成之過氧化氫是由脫落酸所誘導

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鉀為植物生長之必要元素。植物在缺鉀狀況下可形成活化氧族（包括過氧化氫）。證據顯示脫落酸之作用機制與過氧化氫的形成有關。本研究主要是探討水稻幼苗生長在缺鉀的狀況下，葉片內所形成之過氧化氫是否與脫落酸之形成有關。水稻幼苗葉片之過氧化氫係利用組織化學與化學分析法測定。缺鉀確定會造成水稻幼苗葉片過氧化氫之累積。NADPH oxidase 之抑制劑 diphenyleneiodonium chloride 與 imidazole 可降低缺鉀所造成之過氧化氫累積。脫落酸含量則以 *OsRab16A* 基因表現以及免疫法來檢測。缺鉀狀況下亦可造成水稻幼苗葉片脫落酸含量之增加。然而，缺鉀狀況下水稻幼苗葉片內脫落酸之累積不是由過氧化氫所誘導。脫落酸合成抑制劑鎢酸鈉能有效降低缺鉀所引起之脫落酸含量增加，以及過氧化氫之累積。該結果似乎說明缺鉀所形成的過氧化氫是經由脫落酸所誘導。正常狀況下生長之水稻幼苗經由外加脫落酸處理，可增加葉片內過氧化氫含量。此結果更支持脫落酸可誘導水稻缺鉀葉片過氧化氫形成之結論。

關鍵詞：脫落酸；過氧化氫；缺鉀；水稻。