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Hydrogen peroxide is involved in methyl jasmonate-induced senescence of rice leaves

Kuo Tung Hung, Yi Ting Hsu and Ching Huei Kao*

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

Correspondence
*Corresponding author,
e-mail: kaoch@ntu.edu.tw

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The role of H_2O_2 in the senescence of detached rice leaves induced by methyl jasmonate (MJ) was investigated. MJ treatment resulted in H_2O_2 production in detached rice leaves, which was prior to the occurrence of leaf senescence. Dimethylthiourea, a chemical trap of H_2O_2, was observed to be effective in inhibiting MJ-induced senescence and MJ-increased malondialdehyde (MDA) content in detached rice leaves. Diphenyleneiodonium chloride (DPI) and imidazole (IMD), inhibitors of NADPH oxidase, prevented MJ-induced H_2O_2 production, suggesting that NADPH oxidase is a H_2O_2-generating enzyme in MJ-treated detached rice leaves. DPI and IMD also inhibited MJ-promoted senescence and MJ-increased MDA content in detached rice leaves. Phosphatidylinositol 3-kinase inhibitors wortmannin (WM) or LY 294002 (LY) inhibited MJ-induced H_2O_2 production and senescence of detached rice leaves. Exogenous H_2O_2 reversed the inhibitory effect of WM or LY. In terms of leaf senescence, it was observed that rice seedlings of cultivar Taichung Native 1 (TN1) are jasmonic acid (JA)-sensitive and those of cultivar Tainung 67 (TNG67) are JA-insensitive. On treatment with JA, H_2O_2 accumulated in the leaves of TN1 seedlings but not in the leaves of TNG67. Evidence was also provided to show that MJ-induced H_2O_2 production in detached rice leaves is abscisic acid (ABA)-independent. Ethylene action inhibitor, silver thiosulfate, was observed to inhibit MJ- and ABA-induced H_2O_2 production and senescence of detached rice leaves, suggesting that the action of MJ and ABA is ethylene-dependent.

Introduction

Methyl jasmonate (MJ) was first considered as one of many plant secondary metabolites with a possible application in the perfume industry (Demole et al. 1962). It is now becoming evident that jasmonates can act as true plant hormones, which mediate in various aspects of development and stress responses (Creelman and Mullet 1997). It has been shown that jasmonates are powerful promoters of leaf senescence (Ueda and Kato 1981, Weidhase et al. 1987, Chou and Kao 1992, Beltrano et al. 1998, Hung and Kao 1998, 2004a). Recent molecular studies have confirmed that jasmonic acid (JA) may play a role in leaf senescence (He et al. 2002).

Active oxygen species (AOS)-initiated lipid peroxidation has been thought to be an important mechanism of

Abbreviations – ABA, abscisic acid; AOS, active oxygen species; DAB, 3,3-diaminobenzidine; DMTU, dimethylthiourea; DPI, diphenyleneiodonium chloride; ELISA, enzyme-linked immunosorbent assay; FW, fresh weight; IMD, imidazole; LY, LY 294002; MDA, malondialdehyde; MJ, methyl jasmonate; PI3K, phosphatidylinositol 3-kinase; PI3P, phosphatidylinositol 3-phosphate; TN1, Taichung Native 1; TNG67, Tainung 67; WM, wortmannin.

leaf senescence (Kellogg and Fridovich 1975, Thompson et al. 1987). It has been shown that MJ causes the generation of H_2O_2 (Orozco-Cárdenas and Ryan 1999, Orozco-Cárdenas et al. 2001, Hung and Kao 2004a) and lipid peroxidation expressed as malondialdehyde (MDA) production in plant cells (Hung and Kao 1998, 2004a). Thus, MJ leads to oxidative stress in plant cells.

H_2O_2 is a constituent of oxidative metabolism and is itself an AOS. It has been shown that H_2O_2 promotes leaf senescence (Begam and Choudhuri 1992, Lin and Kao 1998) and induction of senescence is accompanied by an increase in endogenous H_2O_2 content (Mondal and Choudhuri 1981, Hung and Kao 2003, 2004a). Because H_2O_2 is relatively stable and diffusible through membrane, H_2O_2 is thought to constitute a general signal molecule inducing cellular stress (Foyer et al. 1997, Neill et al. 2002). Thermoprotection obtained by spraying salicylic acid or by heat acclimation was suggested to be achieved by a common signal transduction pathway involving very early increase in H_2O_2 content (Dat et al. 1998). In tomato plants, H_2O_2 has been shown to act as a second messenger for the induction of defense genes in response to wounding, systemin, and MJ (Orozco-Cárdenas et al. 2001). MJ has also been shown to generate H_2O_2 in parsley suspension-cultured cells (Kauss et al. 1994). It has been demonstrated that H_2O_2 is required for the induction of rice cytosolic ascorbate peroxidase mRNA (Morita et al. 1999). H_2O_2 has now also been shown to be a crucial component of abscisic acid (ABA)-induced stomatal closure (Pei et al. 2000, Zhang et al. 2001, Kwak et al. 2003) and gibberellic acid-induced programmed cell death in the aleurone cells of barley (Fath et al. 2001).

We have previously shown that MJ not only increases the contents of H_2O_2 but also causes protein loss (senescence) and lipid peroxidation in rice leaves (Hung and Kao 2004a). These results suggest that MJ causes oxidative stress and MJ-promoted senescence is mediated through oxidative stress. In this article, we have examined the role of H_2O_2 as a link between MJ and subsequent senescence in detached rice leaves.

Materials and methods

Plant materials

Rice (*Oryza sativa* L., cv. Taichung Native 1, TN1, or Tainung 67, TNG67) 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 paper at 37°C under dark conditions. After 48-h incubation, uniformly germinated seeds were selected and cultivated in a 500-ml beaker containing half-strength Kimura B solution as described previously (Hsu and Kao 2005). The hydroponically cultivated seedlings were grown for 12 days in a Phytotron (Agricultural Experimental Station, National Taiwan University, Taipei, Taiwan) with natural sunlight at 30°C day/25°C night and 90% relative humidity. The apical 3 cm of the third leaf of TN1 was used in experiments. A group of 10 segments was floated in a Petri dish containing 10 ml of test solution. Incubation was carried out at 27°C in the dark. For experiments of intact leaves of TN1 and TNG67 seedlings, JA was added to half-strength Kimura B solution at the time when the third leaf was fully expanded.

Determinations of protein, H_2O_2, lipid peroxidation, and ABA

The senescence of detached rice leaves was followed by measuring the decrease of protein content. For protein extraction, leaf segments were homogenized in 50 m*M* sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17 600 *g* for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976).

The H_2O_2 content was measured colorimetrically as described by Jana and Choudhuri (1982). H_2O_2 was extracted by homogenizing leaf tissue with phosphate buffer (50 m*M*, pH 6.5) containing 1 m*M* hydroxylamine. The homogenate was centrifuged at 6000 *g* for 25 min. To determine H_2O_2 content, the extracted solution was mixed with 0.1% titanium chloride in 20% (v/v) H_2SO_4. The mixture was then centrifuged at 6000 *g* for 25 min. The absorbance was measured at 410 nm. Using this method, we obtained that absorbance increased linearly with the amount of H_2O_2 and addition of H_2O_2 to extracts resulted in the predicted increase of absorbance, i.e. added H_2O_2 was fully recovered (data not shown). The H_2O_2 content in leaf extracts was calculated using the extinction coefficient of 0.28 $\mu mol^{-1}\ cm^{-1}$. In some experiments, H_2O_2 was also visually detected in the leaves by using 3,3-diaminobenzidine (DAB) as substrate (Orozco-Cárdenas and Ryan 1999). Detached rice leaves were supplied through the cut ends with DAB (1 mg ml^{-1}) solution for 24 h under light at 27°C. Leaves were then decolorized in boiling ethanol (95%) for 0.5 h. This treatment decolorized the leaves except for the brown polymerization product produced by DAB with H_2O_2. After cooling, the leaves were extracted at room temperature with fresh ethanol. The H_2O_2 staining was repeated four times with similar results.

MDA, routinely used as an indicator of lipid peroxidation, was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer (1968).

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 polyvinylpyrrolidone column and C18 (Sep-Pak®· Vac) cartridges (Waters, Milford, MA). 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 (Walker-Simmons 1987). ABA immunoassay detection kit (PGR-1) purchased from Sigma Chemical Co. (St Louis, MO) is specific to (+)–ABA. By evaluating ^{3}H-ABA recovery, ABA loss was <3% by the method described here.

Preparation of silver thiosulfate

A stock solution of silver thiosulfate (STS) was prepared by mixing equal volume of 0.01 *M* $AgNO_3$ and 0.04 *M* $Na_2S_2O_3$ (Liu et al. 1990).

Statistical analysis

Statistical differences between measurements ($n = 4$) on different treatments or on different times were analyzed following the Duncan's multiple range test or Student's *t*-test.

Results

MJ increases H_2O_2 production and promotes senescence

Senescence of detached rice leaves in the present study was followed by measuring the decrease of protein. MDA is used as an indicator of lipid peroxidation. The changes in protein and MDA contents in detached rice leaves treated with 45 μ*M* MJ in the dark are shown in Fig. 1A, B. The decrease in protein content and increase in MDA content were evident 24 h after MJ treatment. Clearly, MJ is effective in promoting senescence and causing lipid peroxidation. Lipid peroxidation is caused by AOS (Kellogg and Fridovich 1975, Thompson et al. 1987). MJ also caused an increase in H_2O_2 content (Figs 1C and 2). The increase in H_2O_2 was evident 12 h after treatment of MJ, which was prior to the decrease in protein and increase in MDA. These results suggest that H_2O_2 may play an important role in regulating the senescence of rice leaves induced by MJ.

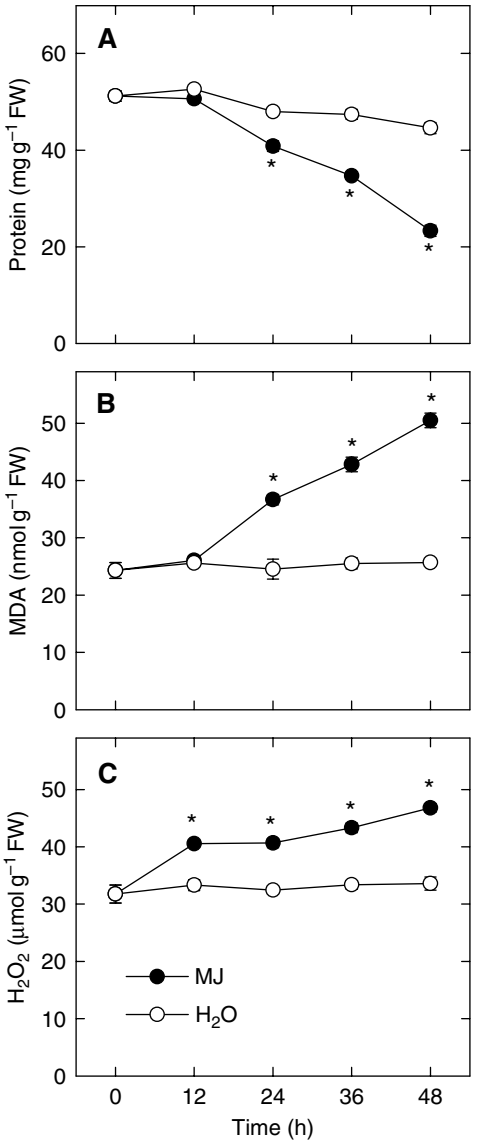

Fig. 1. Changes in the contents of protein (A), malondialdehyde (MDA) (B), and H_2O_2 (C) in detached rice leaves treated with methyl jasmonate (MJ). Detached rice leaves were treated with either water or 45 μ*M* MJ in the dark. Values are mean ± SE (n = 4). The missing error bars indicate that they are smaller than the label marks. Asterisk represents values that are significantly different between H_2O and MJ treatments at $P < 0.05$ level by Student's *t*-test.

The effect of dimethylthiourea, a chemical trap for H_2O_2

To demonstrate the involvement of H_2O_2 in the effects induced by MJ in detached rice leaves, namely the decrease in protein content and the increase in MDA content, dimethylthiourea (DMTU), a chemical trap for H_2O_2 (de Agazio and Zacchini 2001), was used. Detached rice leaves were incubated in a solution

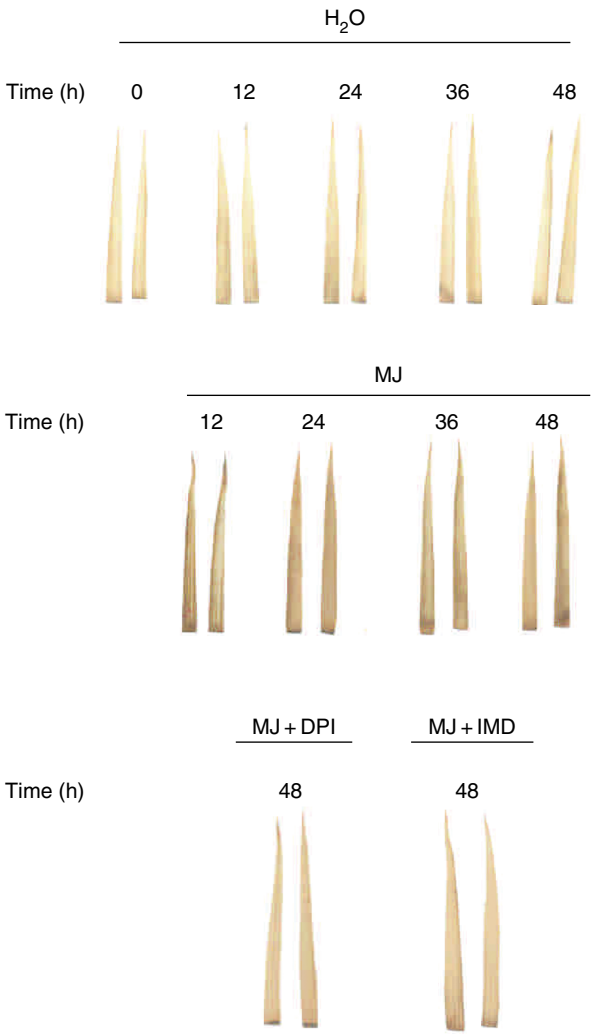

Fig. 2. Histochemical detection of H_2O_2 with DAB staining in detached rice leaves treated with water, methyl jasmonate (MJ), and MJ plus diphenyleneiodonium chloride (DPI) or imidazole (IMD). The concentrations of MJ, DPI, and IMD were 45, 1, and 100 μ*M*, respectively. DAB, 3,3-diaminobenzidine.

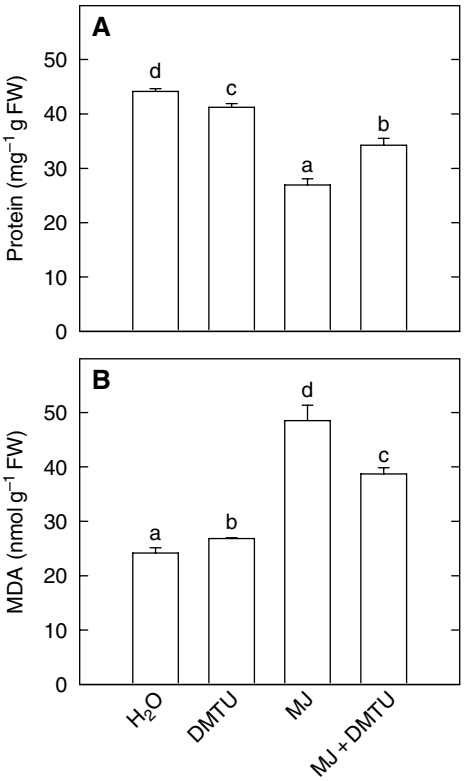

Fig. 3. Effect of dimethylthiourea (DMTU) on the contents of protein (A) and malondialdehyde (MDA) (B) in detached rice leaves treated with methyl jasmonate (MJ). The concentrations of MJ and DMTU were 45 μ*M* and 5 m*M*, respectively. All measurements were determined 2 days after treatment in the dark. Values are mean ± SE (n = 4). Values with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

containing MJ (45 μ*M*) with or without DMTU (5 m*M*). The decrease in protein and the increase in MDA in rice leaves caused by MJ were reduced by DMTU (Fig. 3A, B).

The effect of NADPH oxidase inhibitors

AOS, originating from the plasma membrane NADPH oxidase, which transfers electrons from cytoplasmic NADPH to O_2 to form $O_2^{-}\cdot$, followed by dismutation of $O_2^{-}\cdot$ to H_2O_2, has been a recent focus in AOS signaling. There are reports, indicating that the oxidative burst and the accumulation of H_2O_2 appear to be mediated by the activation of plasma membrane NADPH oxidase complex in plants (Ogawa et al. 1997, del Río et al. 1998, Potikha et al. 1999, Jiang and Zhang 2002). Some chemical inhibitors of the NADPH oxidase complex found in mammalian neutrophils, such as diphenyleneiodonium chloride (DPI) and imidazole (IMD), inhibit the pathogen-, elicitor-, wound-, and abscisic acid-induced accumulation of H_2O_2 in plants (Levine et al. 1994, Auh and Murphy 1995, Bestwick et al. 1997, Alvarez et al. 1998, Orzco-Cárdenas and Ryan 1999, Jiang and Zhang 2002). When detached rice leaves were treated with a solution of DPI (1 μ*M*) or IMD (100 μ*M*), MJ-induced accumulation of H_2O_2 in rice leaves was reduced (Figs 2 and 4C). DPI or IMD also inhibited MJ-promoted leaf senescence (Fig. 4A) and MJ-increased contents of MDA (Fig. 4B).

The effect of phosphatidylinositol 3-kinase inhibitors

Recently, Jung et al. (2002) and Park et al. (2003) suggested that phosphatidylinositol 3-phosphate

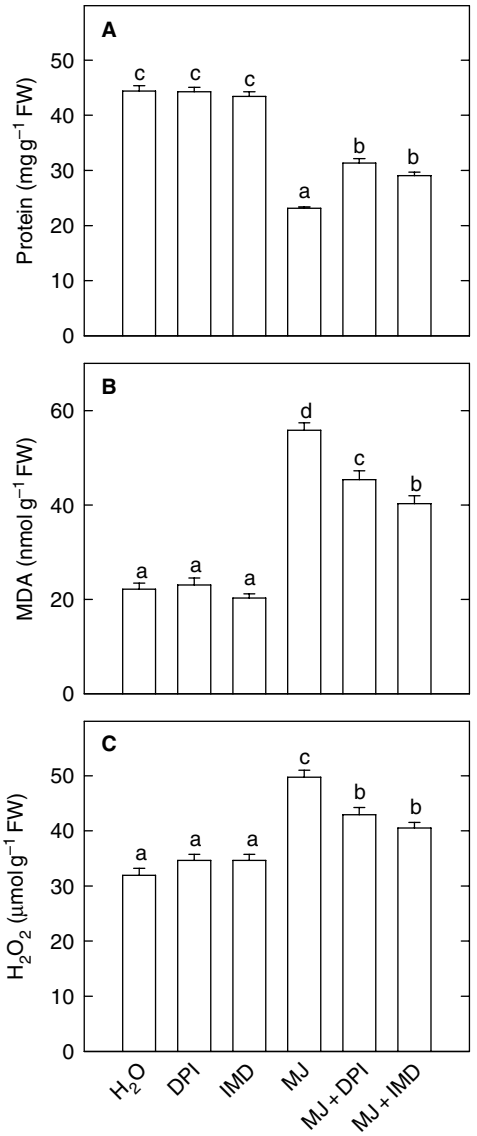

Fig. 4. Effect of diphenyleneiodonium chloride (DPI) and imidazole (IMD) on the contents of protein (A), malondialdehyde (MDA) (B), and H_2O_2 (C) in detached rice leaves treated with methyl jasmonate (MJ). The concentrations of MJ, DPI, and IMD were 45, 1, and 100 μ*M*, respectively. All measurements were determined 2 days after treatment in the dark. Values are mean ± SE (n = 4). Values with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

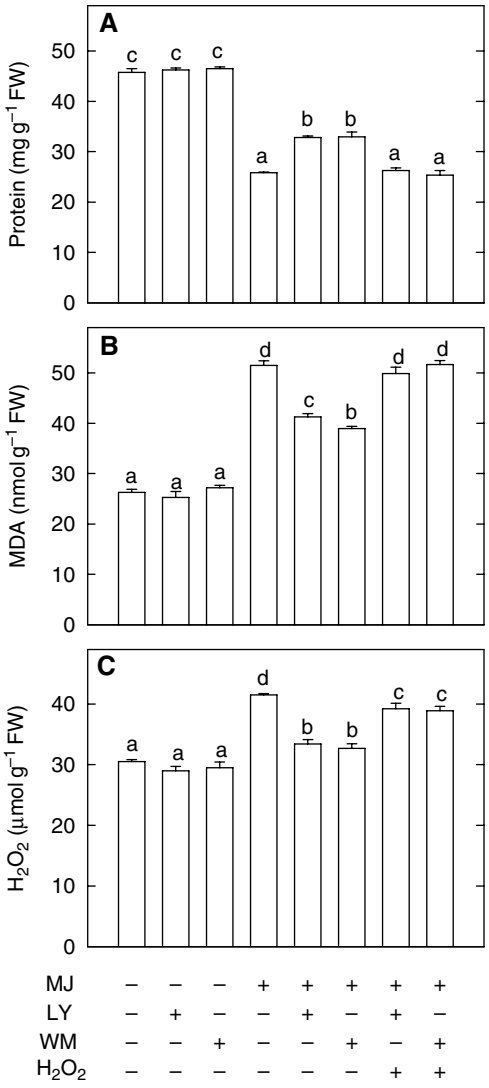

Fig. 5. Effect of LY 294002 (LY) and wortmannin (WM) on the contents of protein (A), malondialdehyde (MDA) (B), and H_2O_2 (C) in methyl jasmonate (MJ)-treated rice leaves in the presence or absence of hydrogen peroxide. The concentrations of LY, WM, MJ, and hydrogen peroxide were 100 μ*M*, 1 μ*M*, 45 μ*M*, and 1 m*M*, respectively. All measurements were determined 2 days after treatment in the dark. Values are mean ± SE (n = 4). Values with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

(PI3P) is important in NADPH oxidase-mediated H_2O_2 production during ABA-induced stomatal closure. Thus, it is of great interest to understand whether PI3P is also important in MJ-induced H_2O_2 production and senescence of rice leaves. LY 294002 (LY) and wortmannin (WM) are inhibitors of phosphatidylinositol 3-kinase (PI3K), a product of which is PI3P. When detached rice leaves were treated with a solution of LY (100 μ*M*) or WM (1 μ*M*), MJ-induced accumulation of H_2O_2 in detached rice leaves was reduced (Fig. 5C). LY or WM also inhibited MJ-promoted senescence (Fig. 5A) and MJ-increased MDA content (Fig. 5B). Exogenous H_2O_2 (1 m*M*) was observed to be able to reverse the inhibitory effects of LY or WM on MJ-induced senescence and lipid peroxidation (Fig. 5A, B).

JA induces H_2O_2 accumulation in the leaves of cultivar TN1 seedlings but not in cultivar TNG67

Figure 6 shows the effect of JA concentrations, in the range from 5 to 40 μ*M*, applied over a period of 3 days, on the senescence of the second leaves of rice seedlings. It is clear that increasing concentration of JA progressively promotes senescence of the second leaves of TN1, but not in TNG67 seedlings. It appears that in terms of leaf senescence, TNG67 is JA-insensitive and TN1 is JA-sensitive. If H_2O_2 is important in regulating rice leaf senescence, then H_2O_2 content is expected to increase in JA-treated TN1 seedlings but not in TNG67. As indicated in Fig. 7 (A, C), it is indeed the case. It is also clear from Fig. 7 (B, D) that JA increased MDA content in the second leaves of TN1 but not in TNG67.

ABA but not MJ increases endogenous ABA content in detached rice leaves

In our recent work, we reported that H_2O_2 is necessary for ABA-induced senescence of detached rice leaves (Hung and Kao 2004b). To determine whether MJ-induced H_2O_2 production and MJ-promoted senescence of detached rice leaves are mediated through an accumulation of ABA, we measured endogenous ABA content in ABA- and MJ-treated detached rice leaves. It is clear that ABA but not MJ treatment caused an increase in the content of endogenous ABA in detached rice leaves (Fig. 8). It appears that MJ-induced H_2O_2 production and MJ-promoted senescence in detached rice leaves are ABA-independent.

An increase in ethylene sensitivity is associated with MJ- and ABA-increased H_2O_2 production in detached rice leaves

A change in ethylene sensitivity in MJ- or ABA-treated detached rice leaves was tested by using STS, an inhibitor of ethylene action. It was observed that treatment of detached rice leaves with STS inhibited the increase in H_2O_2 and MDA contents (Fig. 9A, B) and the decrease in protein content (Fig. 9C) caused by MJ and ABA, suggesting that ethylene action is required for ABA- and MJ-induced H_2O_2 production.

Discussion

It has been shown that MJ is able to generate H_2O_2 in parsley suspension-cultured cells (Kauss et al. 1994) and tomato leaves (Orozco-Cárdenas and Ryan 1999, Orozco-Cárdenas et al. 2001). Here, we also show that MJ induced H_2O_2 production in rice leaves (Figs 1C and 2). Wounding is known to induce H_2O_2 production (Orozco-Cárdenas and Ryan 1999). When detached rice leaves are used to study senescence, wounding is always a problem. However, in the present study, each long and narrow rice leaf was cut

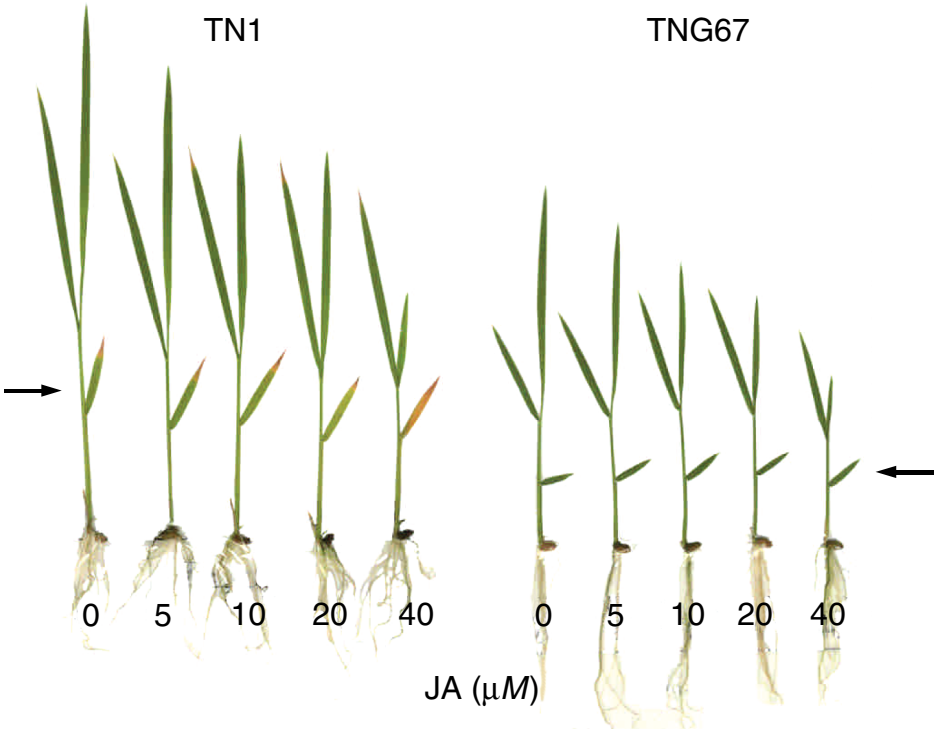

Fig. 6. Effect of jasmonic acid (JA) on the senescence of the second leaves of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings. Rice seedlings were cultivated in half-strength Kimura B solution in a Phytotron with natural sunlight at 30°C day/25°C night and 90% relative humidity. JA was added to half-strength Kimura B solution at the time when the third leaves were fully expanded. Arrows indicate the second leaves. Pictures were taken 3 days after adding JA.

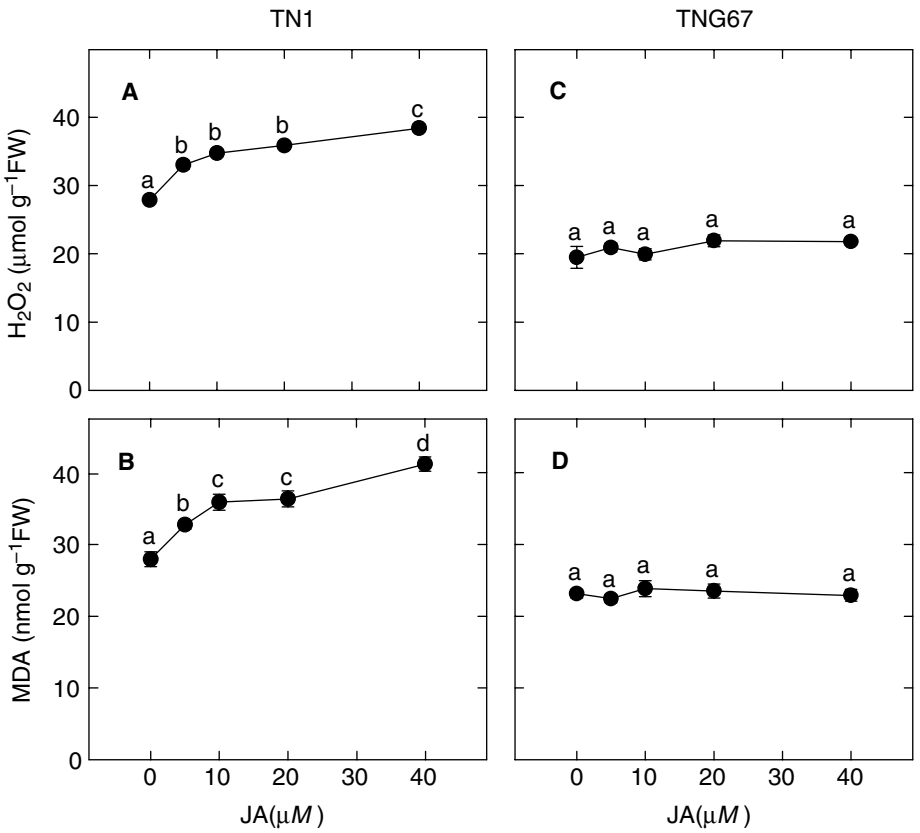

Fig. 7. Effect of jasmonic acid (JA) on the contents of H_2O_2 (A and C) and malondialdehyde (MDA) (B and D) in the second leaves of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings. Rice seedlings were cultivated in half-strength Kimura B solution in a Phytotron with natural sunlight at 30°C day/25°C night and 90% relative humidity. JA was added to half-strength Kimura B solution at the time when the third leaves were fully expanded. H_2O_2 and MDA contents were determined 3 days after adding JA. Values are mean ± SE (n = 4). Values with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

transversely; thus, the area of wounding was very small. Therefore, H_2O_2 generation of detached rice leaves induced by MJ is unlikely to be complicated by the wounding effect.

A role for plasma membrane NADPH oxidase in the generation of the H_2O_2 has been a recent focus in AOS signaling. Here, we show that DPI and IMD, inhibitors of NADPH oxidase (Levine et al. 1994, Auh and Murphy 1995, Bestwick et al. 1997, Alvarez et al. 1998, Orozco-Cárdenas et al. 2001, Jiang and Zhang 2002, Kwak et al. 2003), reduced MJ-induced H_2O_2 production (Fig. 5C) and lipid peroxidation (Fig. 5B), and MJ-promoted senescence (Fig. 5A) in rice leaves. Similar results were obtained by using DMTU, a chemical trap for H_2O_2 (Fig. 3). Furthermore, the increase in H_2O_2 content by MJ was observed to precede the occurrence of leaf senescence and lipid peroxidation (Fig. 1). It appears that H_2O_2 is involved in MJ-induced senescence of detached rice leaves. In the present study, JA is effective in inducing senescence of the leaves of TN1 rice seedlings but not in TNG67, suggesting that in terms of senescence, TN1 is JA-sensitive and TNG67 is JA-insensitive (Fig. 6). The fact that on treatment with JA, the H_2O_2 content increased in JA-sensitive TN1 seedling leaves but not in JA-insensitive TNG67 (Fig. 7) supports further that H_2O_2 is involved in leaf senescence caused by jasmonates.

It has been shown that a high concentration of DPI can affect other enzymes potentially involved in the generation of AOS, including cell wall peroxidase and nitric oxide synthase (Bolwell et al. 1998). The fact that MJ-induced H_2O_2 accumulation in detached rice leaves can be inhibited by low concentration (1 μ*M*) of DPI and can be inhibited by both DPI and IMD (Figs 2 and 4C) strongly suggests that MJ-dependent H_2O_2 generation originated, at least in part, from plasma membrane NADPH oxidase.

The mechanism of AOS production and the molecules involved have been well investigated in animal cells, particularly in neutrophils. The NADPH oxidase complex, which consists of many components, is responsible for AOS production in neurophil cells and is activated by the binding of PI3P to one of the components (Ellson et al. 2001). It appears that PI3P is important in MJ-induced H_2O_2 production based on two lines of evidence. First, LY or WM, inhibitors of PI3K, was able to reduce MJ-induced H_2O_2 production, senescence, and lipid peroxidation (Fig. 5). Second, exogenous H_2O_2 reversed the inhibitory effect of the PI3K

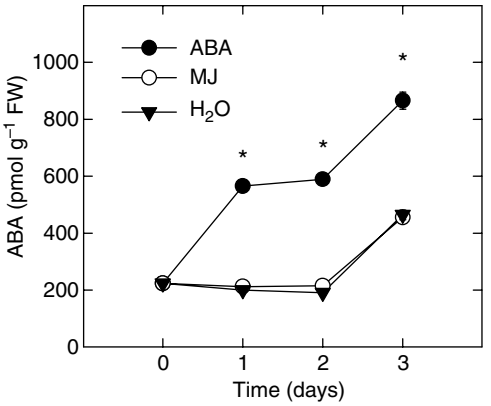

Fig. 8. Changes in the contents of endogenous abscisic acid (ABA) in detached rice leaves treated with methyl jasmonate (MJ) and ABA. The concentrations of MJ and ABA were 45 μ*M*. Values are mean ± SE (n = 4). The missing error bars indicate that they are smaller than the label marks. Asterisk represents values that are significantly different between H_2O and ABA treatments at $P < 0.05$ level by Student's *t*-test when compared to water control.

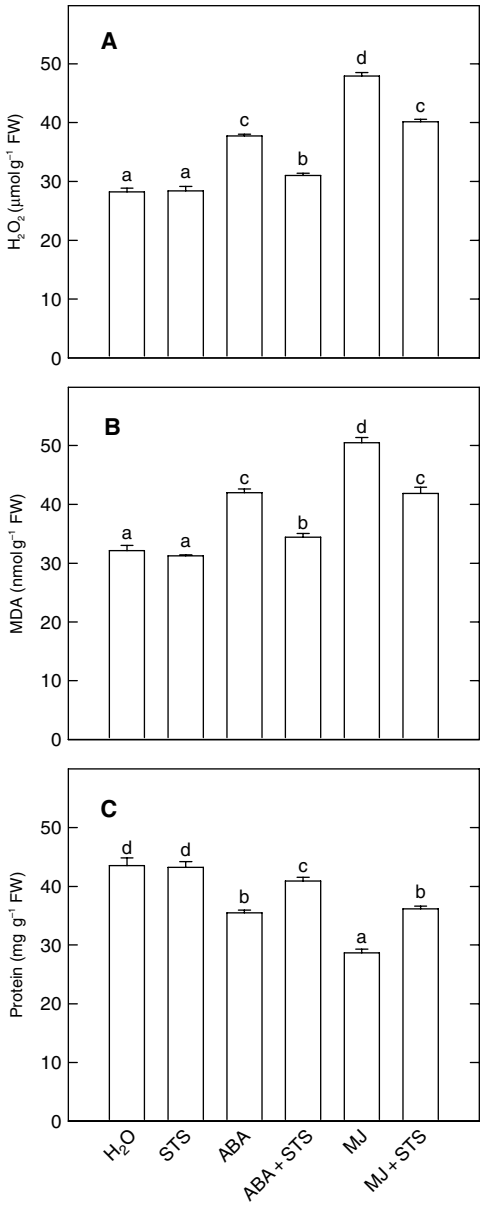

Fig. 9. Effect of silver thiosulfate (STS) on the contents of H_2O_2 (A), malondialdehyde (MDA) (B), and protein (C) of detached rice leaves in the presence or absence of abscisic acid (ABA) and methyl jasmonate (MJ). The concentrations of STS, MJ, and ABA were 200, 45, and 45 μ*M*, respectively. All measurements were determined 2 days after treatment in the dark. Values are mean ± SE (n = 4). Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

inhibitors on the MJ-induced H_2O_2 accumulation, senescence, and lipid peroxidation. These results supported further the conclusion that H_2O_2 is involved in MJ-induced senescence of detached rice leaves and that NADPH oxidase is involved in MJ-induced H_2O_2 production. PI3P has also been shown to be important in the ABA-induced H_2O_2 generation in guard cells (Jung et al. 2002, Park et al. 2003). Our recent observation also demonstrated that PI3P is required for ABA-induced H_2O_2 production in detached rice leaves (Hung and Kao 2005). In neutrophils, PI3P regulated H_2O_2 production by binding to the non-catalytic component $p40^{phox}$ of the NADPH oxidase (Ellson et al. 2001). However, a rice homolog of $p40^{phox}$ has not been reported. Therefore, the detailed mechanism of the action of PI3P during H_2O_2 production in the rice leaves awaits further investigation.

Recently, a cell wall peroxidase has been identified in French bean (Bolwell et al. 1998, Blee et al. 2001), and a potentially peroxidase-mediated H_2O_2 production has been demonstrated in *Arabidopsis* cultures challenged with a fungal elicitor (Bolwell et al. 2002). As DPI and IMD did not completely inhibit MJ-induced H_2O_2 production (Figs 2 and 4C), peroxidase may be another H_2O_2-generating enzyme operating in MJ-treated rice leaves.

In plants, polyamines are known to play an important role in growth, development, and stress responses (Walden et al. 1997). It has been shown that H_2O_2 produced by diamine or polyamine oxidase induced hypersensitive cell death in plants (Yoda et al. 2003). Previously, we have shown that MJ treatment increased putrescine, a diamine, content but had no effect on spermidine and spermine contents in rice leaves (Chen et al. 1994). It appears that diamine or polyamine oxidase is unlikely to be affected by MJ in rice leaves. An alternative source for H_2O_2 generation includes oxalate oxidase, an enzyme that degrades

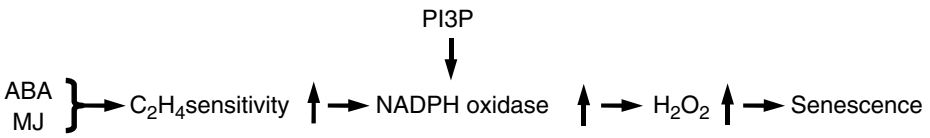

Fig. 10. Regulation of senescence in abscisic acid (ABA)- and methyl jasmonate (MJ)-treated detached rice leaves.

oxalate to CO_2 and H_2O_2 (Dumas et al. 1995). Oxalate oxidase gene expression is induced by salt stress, salicylic acid, and MJ in barley roots (Hurkman and Tanaka 1996). It is not known whether MJ will activate oxalate oxidase in rice leaves. Further work is required to clarify this possibility.

It appears that when detached rice leaves are treated with MJ, H_2O_2 is produced in the apoplast. Because apoplast has only a small proportion of the cell's antioxidant capacity, H_2O_2 will rapidly move into the cell to exert its effect on senescence. It has been suggested that peroxiporins or water channels (aquaporins) may serve as conduits for trans-membrane H_2O_2 transport (Neill et al. 2002). Thus, H_2O_2 can function as a mobile signal in MJ-treated detached rice leaves, but whether H_2O_2 is the sole signal remains to be determined.

Our recent work demonstrated that H_2O_2 is also involved in ABA-induced senescence of rice leaves (Hung and Kao 2004b). Our data revealed that MJ treatment did not result in an accumulation of ABA in detached rice leaves (Fig. 8), suggesting that MJ-induced H_2O_2 production is ABA-independent.

There are reports, showing that ethylene is the major senescence-promoting hormone (Gepstein and Thimann 1982, Kao and Yang 1983, Preger and Gepstein 1985). Several lines of evidence indicated that MJ-promoted leaf senescence was independent of ethylene (Abeles et al. 1989, Cuello et al. 1990). In the present study, we observed that ABA- and MJ-induced H_2O_2 production, lipid peroxidation, and senescence in detached rice leaves are dependent on the ethylene sensitivity (Fig. 9A–C). It appears that in detached rice leaves, one of the earliest events following MJ or ABA treatment is modulating ethylene sensitivity, which then causes the production of H_2O_2 and the subsequent senescence promotion (Fig. 10).

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References

Abeles FB, Hershberg L, Dunn LL (1989) Hormonal and intracellular localization of a 33-kDa cationic peroxidase in excited cucumber cotyledons. Plant Physiol 89: 664–668

de Agazio M, Zacchini M (2001) Dimethylthiourea, a hydrogen peroxide trap, partially prevents stress effects and ascorbate peroxidase increase in spermidine-treated maize roots. Plant Cell Environ 24: 237–244

Alvarez ME, Penell RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92: 773–784

Auh C-K, Murphy TM (1995) Plasma membrane redox enzyme is involved in the synthesis of $O_2^{-}\cdot$ and H_2O_2 by *Phytophthora* elicitor-stimulated rose cells. Plant Physiol 107: 1241–1247

Begam HH, Choudhuri MA (1992) H_2O_2 metabolism during senescence of two submerged angiosperm *Hydrilla* and *Ottelia*: changes in enzyme activities in light and darkness. Biochem Physiol Pflanzen 188: 105–115

Beltrano J, Ronco MG, Montaldi ER, Carbone A (1998) Senescence of flag leaves and ears of wheat hastened by methyl jasmonate. J Plant Growth Regul 17: 53–57

Bestwick CS, Brown IR, Bennett MHR, Mansfield JW (1997) Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Psendomones syringae* pv Phaseolicola. Plant Cell 9: 209–221

Blee KA, Jupe SC, Richard G, Zimmerlin A, Davies DR, Bolwell GP (2001) Molecular identification and expression of the peroxidase responsible for the oxidative burst in French bean (*Phaseolus vulgaris* L.) and related members of the gene family. Plant Mol Biol 47: 607–620

Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Genrrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. J Exp Bot 53: 1367–1376

Bolwell GP, Davies DR, Gerrish C, Auh CK, Murphy TM (1998) Comparative biochemistry of the oxidative burst produced by rose and French bean cells reveals two distinct mechanisms. Plant Physiol 116: 1374–1385

Bradford MM (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

Chen CT, Chou CM, Kao CH (1994) Methyl jasmonate induced accumulation of putrescine but not proline in detached rice leaves. J Plant Physiol 143: 119–121

Chou CM, Kao CH (1992) Methyl jasmonate, calcium, and leaf senescence in rice. Plant Physiol 99: 1693–1694

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48: 355–381

Cuello J, Quiles MJ, Garcia C, Sabater B (1990) Effect of light and growth substances on senescence of barley leaf segments at different developmental stages. Bot Bull Acad Sin 31: 107–112

Dat JF, Foyer CH, Scolt IM (1998) Changes in salicylic acid and antoxidants during induced thermotolerance in mustard seedlings. Plant Physiol 118: 1455–1461

Demole E, Lederer E, Mercier D (1962) Isolement et détermination de la structure du jasmonate de méthyle, constituant odorant charactéristique de léssence de jasmin. Helv Chim Acta 45: 675–685

Dumas B, Freyssinet G, Palett KE (1995) Tissue-specific expression of germin-like oxalate oxidase during development and fungal infection of barley seedlings. Plant Physiol 107: 1091–1096

Ellson CD, Gobert-Gosse S, Anderson KE, Davidson K, Erdjument-Bromage H, Tempst P, Thuring JW, Cooper MA, Lim Z-Y, Holmes AB, Gaffney PRJ, Coadwell J, Chilvers ER, Hawkins PT, Stephens LR (2001) PtdIns (3) P regulates the neutrophil oxidase complex by binding to the PX domain of $p40^{phox}$. Nat Cell Biol 3: 679–682

Fath A, Bethke PC, Jones RL (2001) Enzymes that metabolize reactive oxygen species in barley aleurone cells are downregulated prior to gibberellic acid-induced programmed cell death. Plant Physiol 126: 156–166

Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant 100: 241–254

Gepstein S, Thimann KV (1982) The role of ethylene in the senescence of oat leaves. Plant Physiol 68: 349–354

He Y, Fukushige H, Hildebrand DF, Gan S (2002) Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. Plant Physiol 128: 876–884

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189–198

Hsu YT, Kao CH (2005) Abscisic acid accumulation and cadiunm tolerance in rice seedlings. Physiol Plant 124: 71–80

Hung KT, Kao CH (1998) Involvement of lipid peroxidation in methyl jasmonate-promoted senescence in detached rice leaves. Plant Growth Regul 24: 17–21

Hung KT, Kao CH (2003) Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. J Plant Physiol 160: 871–879

Hung KT, Kao CH (2004a) Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. J Plant Physiol 161: 43–52

Hung KT, Kao CH (2004b) Hydrogen peroxide is necessary for abscisic acid-induced senescence of rice leaves. J Plant Physiol 161: 1347–1357

Hung KT, Kao CH (2005) Phosphatidyl 3-phosphate is required for abscisic acid-induced hydrogen peroxide production in rice leaves. Plant Growth Regul 45: 95–101

Hurkman WJ, Tanaka CK (1996) Effect of salt stress on germin gene expression in barley roots. Plant Physiol 110: 971–977

Jana S, Choudhuri MA (1982) Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat Bot 12: 345–354

Jiang M, Zhang J (2002) Involvement of plasma membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. Planta 215: 1022–1030

Jung J-Y, Kim Y-W, Kwak JM, Hwang J-U, Young J, Schroeder JI, Hwang I, Lee Y (2002) Phosphatidylinositol 3- and 4-phosphate are required for normal stomatal movements. Plant Cell 14: 2397–2412

Kao CH, Yang SF (1983) Role of ethylene in the senescence of detached rice leaves. Plant Physiol 73: 881–885

Kauss H, Jeblick W, Ziegler J, Krabler W (1994) Pretreatment of parsley (*Petroselinum crispum* L.) suspension cultures with methyl jasmonate enhances elicitation of activated oxygen species. Plant Physiol 105: 89–94

Kellogg EW, Fridovich I (1975) Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 25: 8812–8817

Kwak JM, Mori IC, Pei Z-M, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J 22: 2623–2633

Levine A, Tenhaken R, Dixon RA, Lamb CJ (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79: 583–593

Lin JN, Kao CH (1998) Effects of oxidative stress caused by hydrogen peroxide on senescence of rice leaves. Bot Bull Acad Sin 39: 161–165

Liu JH, Mukherjee I, Reid DM (1990) Adventitious rooting in hypocotyls of sunflower (*Helianthus annuus*) seedlings. III. The role of ethylene. Physiol Plant 78: 268–276

Mondal R, Choudhuri MA (1981) Role of hydrogen peroxide in senescence of excised leaves of rice and maize. Biochem Physiol Pflanzen 176: 700–709

Morita S, Kaminaka H, Masumura T, Tanaka K (1999) Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress; the involvement of hydrogen peroxide in oxidative stress signalling. Plant Cell Physiol 40: 417–422

Neill SJ, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. Curr Opin Plant Biol 5: 388–395

Ogawa K, Kanematsu S, Asada K (1997) Generation of superoxide anion and localization of CuZn-superoxide dismutase in the vascular tissue of spinach hypocotyls: their association with lignification. Plant Cell Physiol 38: 1118–1126

Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to

wounding, systemin, and methyl jasmonate. Plant Cell 13: 179–191
Orozco-Cárdenas ML, Ryan CA (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc Natl Acad Sci USA 96: 6553–6557
Park KY, Jung J-Y, Park J, Hwang J-U, Kim Y-W, Hwang I, Lee Y (2003) A role for phosphatidylinositol 3-phosphate in abscisic acid-induced reactive oxygen species generation in guard cells. Plant Physiol 132: 92–98
Pei ZM, Murata N, Benning G, Thomine S, Klüsener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. Nature 406: 731–734
Potikha TS, Collins CC, Johnson DI, Delmer DP, Levine A (1999) The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Plant Physiol 119: 849–858
Preger R, Gepstein S (1985) Regulation of ethylene biosynthesis during senescence of oat leaf segment. Physiol Plant 65: 163–165
del Río LA, Pastori GM, Palma JM, Sandalio LM, Sevilla F, Corpas FJ, Jiménez A, López-Huartas E, Hernández JA (1998) The activated oxygen role of peroxisomes in senescence. Plant Physiol 116: 1195–1200
Thompson JE, Legge RL, Barber RF (1987) The role of free radicals in senescence and wounding. New Phytol 105: 317–344
Ueda J, Kato J (1981) Promotive effect of methyl jasmonate on oat leaf senescence in the light. Z Pflanzenphysiol 103: 357–359
Walden R, Cordeiro A, Tiburcio AF (1997) Polyamines: small molecules triggering pathways in plant growth and development. Plant Physiol 113: 1009–1013
Walker-Simmons M (1987) ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol 84: 61–66
Weidhase RA, Lehmann J, Kramell H, Sembdner G, Parthier B (1987) Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methylester, and counteraction by cytokinins. Physiol Plant 69: 161–166
Yoda H, Yamaguchi Y, Sano H (2003) Induction of hypersensitive cell death by hydrogen peroxide produced through polyamine degradation in tobacco plants. Plant Physiol 132: 1973–1981
Zhang X, Zhang L, Dong F, Gao J, Galbraite DW, Song C-P (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol 126: 1438–1448

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