行政院國家科學委員會專題研究計畫　成果報告

過氧化氫與脫落酸所加速水稻葉片老化間關係之研究

計畫類別：個別型計畫
計畫編號：NSC93-2313-B-002-062-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學農藝學系暨研究所

計畫主持人：高景輝

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 8 月 16 日

行政院國家科學委員會補助專題研究計畫成果報告

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共同主持人：
計畫參與人員：

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中　華　民　國 94 年 8 月 16 日

行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC93-2313-B-002-062

執行期限：93 年 8 月 1 日至 94 年 7 月 31 日

主持人；高景輝　執行機構及單位名稱：台大農藝學系

一、 中文摘要

本研究主要探討脫落酸（ABA）所誘導的水稻切離葉片老化是否經由 H_2O_2 所啟動。ABA 導致水稻切離葉片 H_2O_2 含量增加，此 H_2O_2 含量之增加早於 ABA 所引起之葉片老化。H_2O_2 捕捉劑 dimethylthiourea（DMTU）可有效的抑制 ABA 所引起之葉片老化，malondialdehyde (MDA) 含量之增加，抗氧化酵素（superoxide dismutase, ascorbate peroxidase, glutathione redutase 與 catalase）活性之增加。Diphenyleneiodonium chloride (DPI) 與 imidazole (IMD) [NADPH oxidase 之抑制劑] 以及 KCN 與 NaN_3 [peroxidase 之抑制劑] 亦可阻止 ABA 所誘導 H_2O_2 之含量增加，顯示 NADPH oxidase 與 peroxidase 是負責 ABA 引起形成 H_2O_2 之主要酵素。DPI、IMD、KCN 與 NaN_3 亦可抑制 ABA 所加速之葉片老化，MDA 含量之增加，抗氧化酵素活性之增加以及抗氧化物含量之降低。這些結果說明，H_2O_2 可做為 ABA 所誘導水稻葉片老化之訊息分子。

關鍵詞：脫落酸、過氧化氫、葉片老化、水稻

Abstract

The role of H_2O_2 in abscisic acid (ABA)-induced rice leaf senescence is investigated. ABA treatment resulted in H_2O_2 production in rice leaves, which preceded the occurrence of leaf senescence. Dimethylthiourea, a chemical trap for H_2O_2, was observed to be effective in inhibiting ABA-induced senescence, ABA-induced malondialdehyde (MDA) content, ABA-induced antioxidantive enzyme activities (superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase), and ABA-induced antioxidant content (ascorbic acid and reduced glutathione) in rice leaves. Diphenyleneiodonium chloride (DPI) and imidazole (IMD) [inhibitors of NADPH oxidase], and KCN and NaN_3 [inhibitors of peroxidase] prevented ABA-promoted senescence, ABA-increased MDA contents, ABA-increased antioxidative enzyme activities, and ABA-decreased antioxidant contents in rice leaves. These results suggest that H_2O_2 is involved in ABA-induced senescence of rice leaves.

Keywords: Abscisic acid, Hydrogen peroxide, Leaf senescence, Rice,

二、 緣由與目的

植物荷爾蒙脫落酸（abscisic acid，ABA）可影響許多生理作用與發育過程。ABA 可有效的誘導葉片老化。葉片老化被認為是由於脂質過氧化作用所引起的。活化氧族可啟動脂質過氧化作用。ABA 被認為可誘導活化氧族包括 H_2O_2 之形成，與脂質過氧化作用（即 MDA 之形成）。因此，ABA 可誘導植物細胞之氧化逆境。最近許多研究人員開始重視 H_2O_2 之功能。H_2O_2 也被報告具有加速葉片老化之功用。H_2O_2 是一穩定且能擴散

之分子，因此 H_2O_2 在很多環境逆境上被推測可作為訊息分子。本研究主要的目的在瞭解 ABA 所加速的水稻葉片老化是否經由 H_2O_2 之形成所引起。

三、 結果與討論

利用蛋白質含量之降低做為葉片老化之指標以及 MDA 含量作脂質過氧化作用之指標，我們發現 ABA 可加速水稻葉片老化與脂質過氧化作用 (Figs. 1A, B)，同時我們也發現ABA處理所造成 H_2O_2 之形成早於葉片老化與脂質過氧化作用 (Figs. 1A, 1C)。H_2O_2 之捕捉劑 DMTU 可抑制 ABA 所引起之葉片老化 (Fig. 2A)，MDA 含量增加 (Fig. 2B)，抗氧化酵素（SOD, APOD, GR 與 CAT）活性之增加(Figs. 2C-2F) 以及抗氧化物 AsA (Fig. 3A) 與 GSH (Fig. 3B) 含量之降低。NADPH oxidase 抑制劑 DPI 與 IMD 以及 peroxidase 抑制劑 KCN 與 NaN_3 均可抑制 ABA 所引起 H_2O_2 之形成 (Figs. 4C, 4F, 7C)，顯示 NADPH oxidase 及 peroxidase 為負責 ABA 所引起 H_2O_2 產生之主要酵素。DPI、IMD、KCN 與 NaN_3 亦可有效的阻止 ABA 所引起之葉片老化 (Figs. 4A, 4D, 7A)，MDA 含量之增加 (Figs. 4B, 4E, 7B)，抗氧化酵素活性之增加 (Figs. 5, 6, 8A-8D)，抗氧化物含量之降低 (Figs. 3A, 3B, 8E, 8F)。這些結果充分說明了 H_2O_2 參與 ABA 所誘導之水稻切離葉片老化。

四、 計畫成果與自評

整個計畫執行順利，達到預期之成果。本計畫成果對於 ABA 所誘導水稻切離葉片之機制有進一步明確之瞭解。

五、 參考文獻

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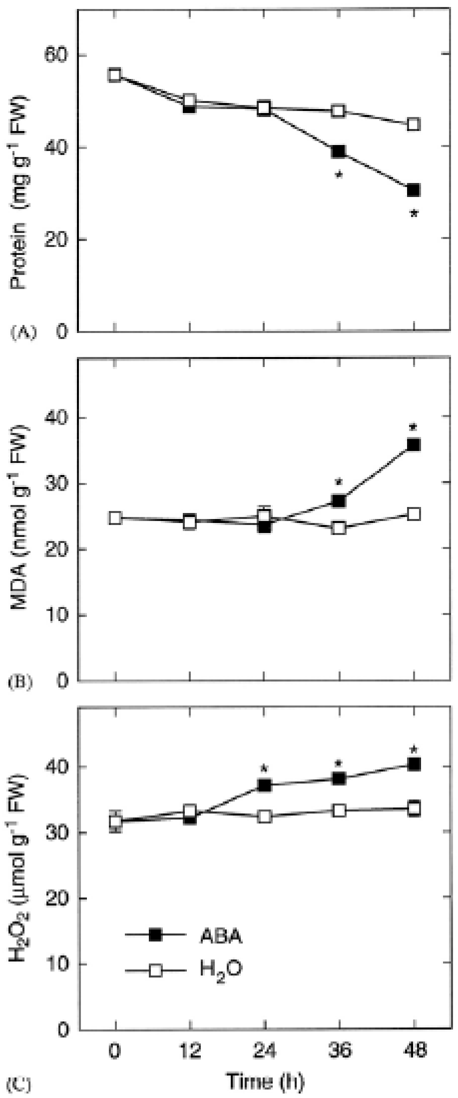

Figure 1. Changes in the contents of protein (A), MDA (B), and H_2O_2 (C) in rice leaves treated with either water or 45 μmol l^{-1} ABA in the dark. Values are means $\pm$ SE ($n = 4$). Asterisks represent values that are significant at $P < 0.05$ level by Student's t-test when compared to water control.

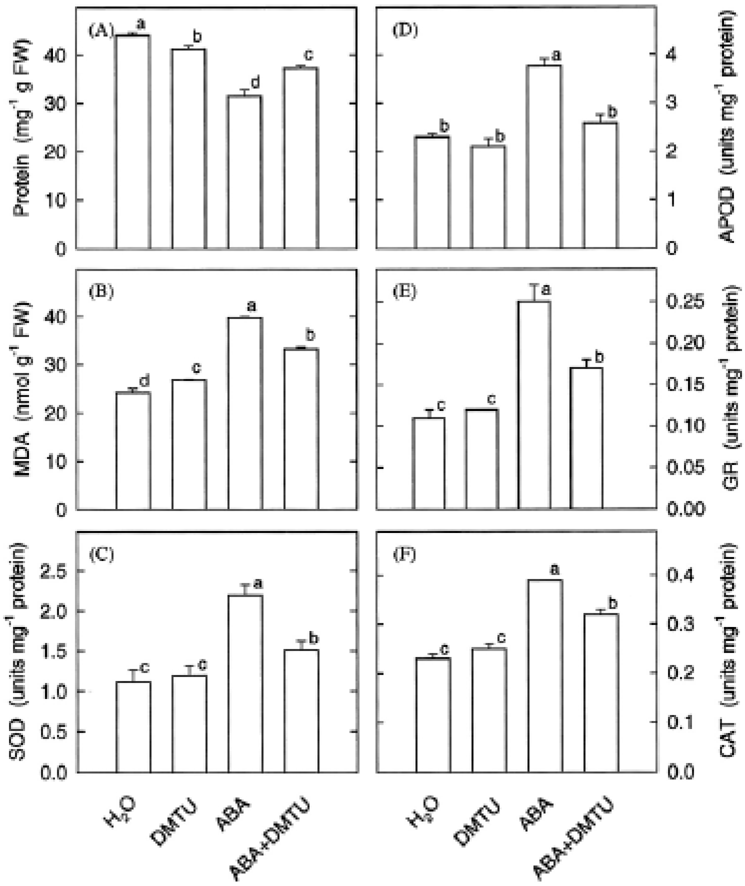

Figure 2. Effect of DMTU on the contents of protein (A), and MDA (B), and the activities of SOD (C), APOD (D), GR (E), and CAT (F) in rice leaves treated with ABA. The concentrations of ABA and DMTU were 45 μmol l^{-1} and 5 mmol l^{-1}, respectively. All measurements were determined 2 days after treatment in the dark. Values are means $\pm$ SE ($n = 4$). Value with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

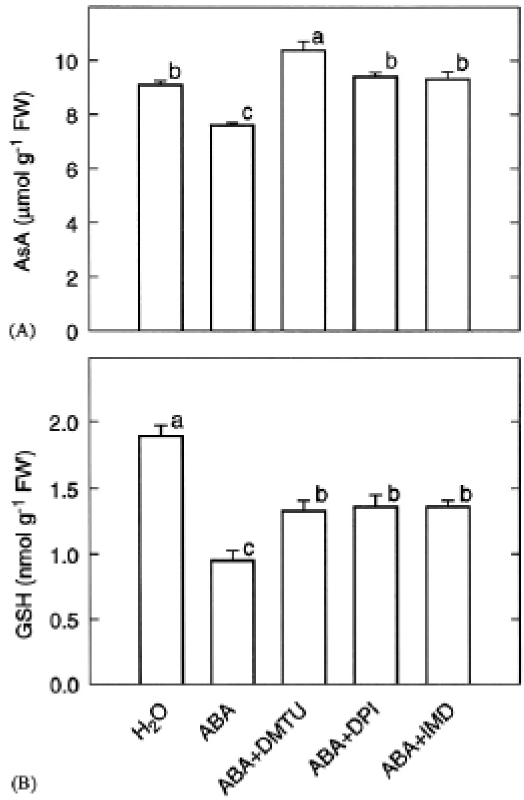

Figure 3. Effect of DMTU, DPI, and IMD on the contents of AsA (A) and GSH (B) in rice leaves treated with ABA. The concentrations of ABA, DMTU, DPI, and IMD were $45\,\mu mol\,l^{-1}$, $5\,mmol\,l^{-1}$, $25\,\mu mol\,l^{-1}$, and $0.1\,mmol\,l^{-1}$, respectively. All measurements were determined 2 days after treatment in the dark. Values are means$\pm$SE ($n = 4$). Value with the same letter are not significantly different at $P<0.05$ level, according to Duncan's multiple range test.

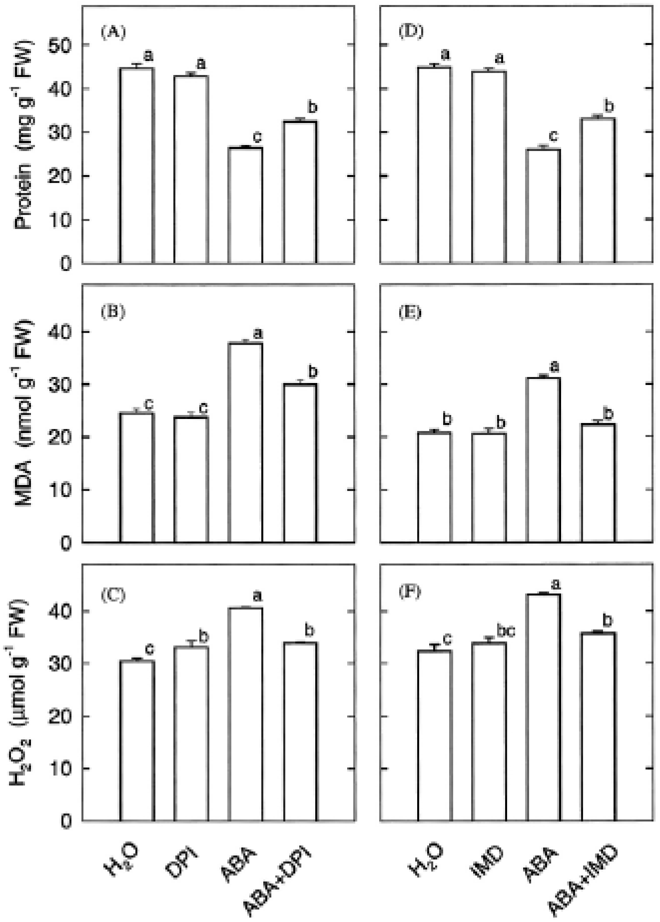

Figure 4. Effect of DPI and IMD on the contents of protein (A, D), and MDA (B, E), and H_2O_2 (C, F) in rice leaves treated with ABA. The concentrations of ABA, DPI, and IMD were 45, 25 $\mu mol\,l^{-1}$, and 0.1 $mmol\,l^{-1}$, respectively. All measurements were determined 2 days after treatment in the dark. Values are means $\pm$ SE ($n = 4$). Value with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

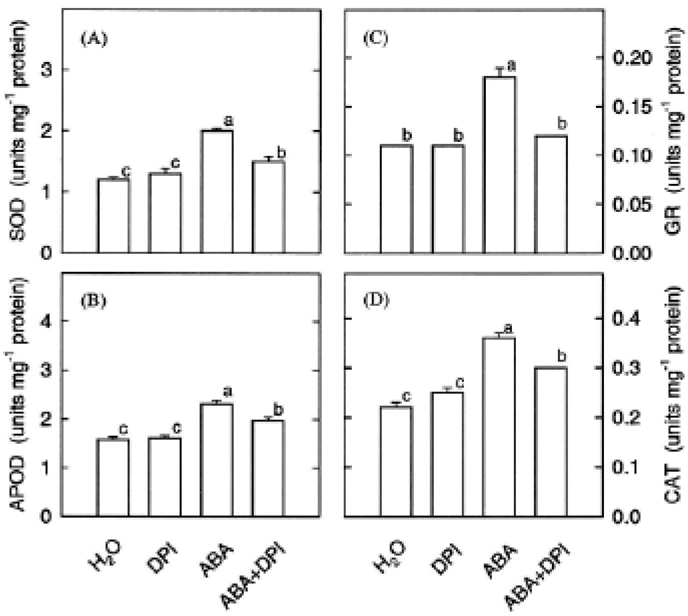

Figure 5. Effect of DPI on the activies of SOD (A), APOD (B), GR (C), and CAT (D) in rice leaves treated with ABA. The concentrations of ABA and DPI were 45 and 25 $\mu mol\,l^{-1}$, respectively. All measurements were determined 2 days after treatment in the dark. Values are means $\pm$ SE ($n = 4$). Value with the same letter are not significantly different at $P<0.05$ level, according to Duncan's multiple range test.

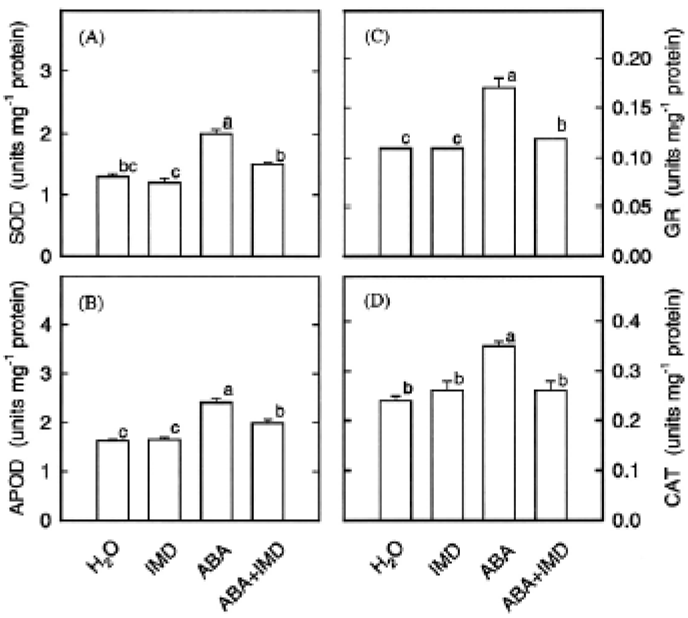

Figure 6. Effect of IMD on the activies of SOD (A), APOD (B), GR (C), and CAT (D) in rice leaves treated with ABA. The concentrations of ABA and IMD were 45 $\mu mol\,l^{-1}$ and 0.1 $mmol\,l^{-1}$, respectively. All measurements were determined 2 days after treatment in the dark. Values are means $\pm$ SE ($n = 4$). Value with the same letter are not significantly different at $P<0.05$ level, according to Duncan's multiple range test.

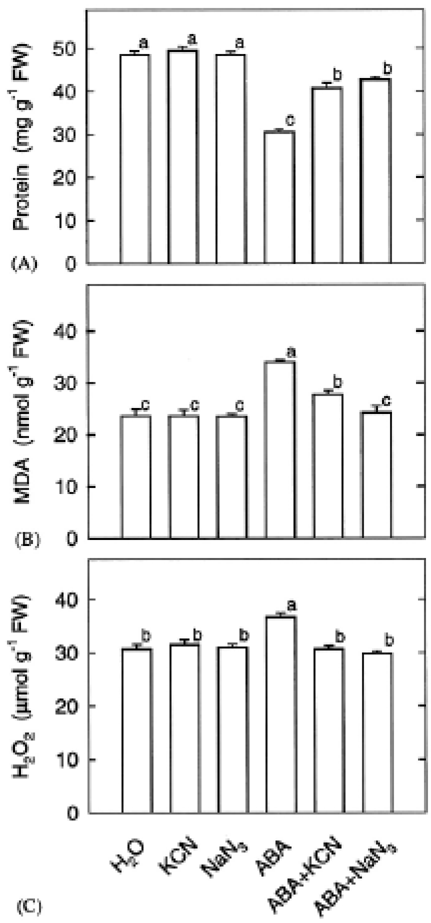

Figure 7. Effect of KCN and NaN_3 on the contents of protein (A), and MDA (B), and H_2O_2 (C) in rice leaves treated with ABA. The concentrations of ABA, KCN, and NaN_3 were 45, 10 µmol l^{-1}, and 1 mmol l^{-1}, respectively. All measurements were determined 2 days after treatment in the dark. Values are means ± SE ($n = 4$). Value with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.

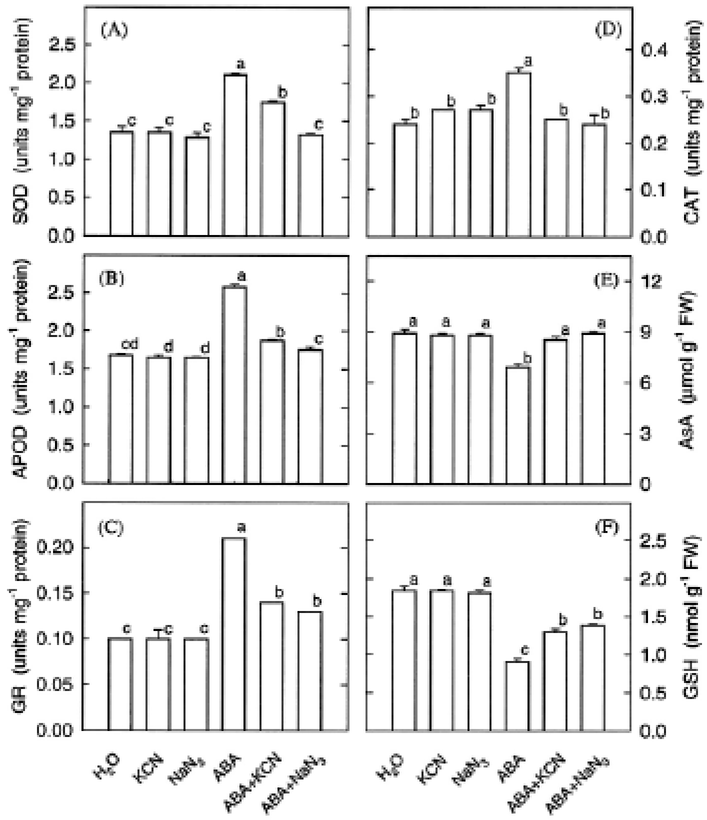

Figure 8. Effect of KCN and NaN_3 on the activies of SOD (A), APOD (B), GR (C), and CAT (D) and the contents of AsA (E) and GSH (F) in rice leaves treated with ABA. The concentrations of ABA, KCN, and NaN_3 were 45, 10 $\mu mol\, l^{-1}$, and 1 $mmol\, l^{-1}$, respectively. All measurements were determined 2 days after treatment in the dark. Values are means $\pm$ SE ($n = 4$). Value with the same letter are not significantly different at $P < 0.05$ level, according to Duncan's multiple range test.