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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※　　一氧化氮對水稻葉片氧化性傷害之研究　　※
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計畫類別：☑個別型計畫　　□整合型計畫
計畫編號：NSC 90－2313－B－002－267－
執行期間：90 年　8 月　1 日至 91 年　7 月 31 日

計畫主持人：高景輝

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執行單位：國立台灣大學農藝系

中　華　民　國　91 年　8　月　1　日

行政院國家科學委員會專題研究計畫成果報告

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一、中文摘要

本研究主要在探討一氧化氮(nitric oxide, NO)對 paraquat、脫水、polyethylene glycol (PEG)、sorbitol、abscisic acid (ABA)、methyl jasmonate (MJ)與鎘所造成之水稻葉片毒害或老化加速之影響。NO 釋放劑可克服 paraquat、脫水、PEG、ABA、MJ 與鎘所造成之毒害或老化加速及脂質過氧化作用。NO 專一性之清除劑 c-PTIO 可使 NO 釋放劑之作用喪失，顯示 NO 釋放劑之作用確實是由所釋放出之 NO 所造成。雖然 sorbitol 處理可加速水稻葉片之老化，但不能增加脂質過氧化作用，NO 釋放劑也不能克服 sorbitol 所加速之水稻葉片老化。

關鍵詞：離層酸、鎘、葉片老化、茉莉酸甲酯、一氧化氮、巴拉刈、水稻、缺水逆境

Abstract

In the present study, we evaluate the effect of nitric oxide (NO) against toxicity or senescence of rice leaves caused by paraquat, dehydration, polyethylene glycol (PEG), sorbitol, abscisic acid (ABA), methyl jasmonate (MJ) and excess Cd. NO donors were effective in inhibiting toxicity or senescence and the increase in lipid peroxidation of paraquat-, dehydration-, PEG-, ABA-, MJ- and Cd-treated rice leaves. The protective effect of NO donors on toxicity or promotion of senescence, and the increase in lipid peroxidation by paraquat, dehydration, PEG, ABA, MJ and Cd was reversed by c-PTIO, a NO specific scavenger, suggesting that the protective effect of NO donor is attributed to NO released. However, the promotion of rice leaf senescence caused by sorbitol, which has no effect on lipid peroxidation, cannot be counteracted by NO-donors.

Keywords: Abscisic acid, Cadmium, Leaf senescence, Methyl jasmonate, Nirtic oxide, Paraquat, Rice, Water deficit

二、緣由與目的

一氧化氮(nitric oxide, NO)是一種不穩定之氣態自由基分子。在動物系統中，許多報告指出，NO 可減緩氧化逆境(或活化氧族)所帶來之傷害。植物遭受逆境時，常會導致氧化逆境，NO 是否亦能克服植物所遭受之氧化逆境傷害，則並不清楚。paraquat 是一種可以產生活化氧族之殺草劑，因此我們希望瞭解 NO 是否可減緩或克服 paraquat 對水稻葉片所造成之氧化傷害。本研究室過去之研究中發現，脫水、polyethylene glycol (PEG)、abscisic acid (ABA)、methyl jasmonate (MJ)以及過量鎘處理所加速之水稻葉片老化（或毒害），均需活化氧族之參與。因此，NO 是否亦可減緩或克服脫水、PEG、ABA、MJ 與過量鎘所加速之葉片老化（或毒害），也是我們的研究重點。

三、結果與討論

paraquat 是一種殺草劑，它會將光合成系統 I 之電子截取傳送給氧分子，產生活

化氧族，而導致過氧化作用與葉片之傷害。我們過去之研究也瞭解，paraquat 在光線下確實會造成水稻葉片氧化傷害。利用這個系統，我們發現 NO 釋放劑可克服 paraquat 所造成之傷害、脂質過氧化作用及抗氧化酵素活性之降低。而 NO 清除劑(c-PTIO)可使 NO 釋放劑之作用消失，顯示 NO 釋放劑之作用確實是由 NO 釋放出來所造成之作用。NO 之克服 paraquat 傷害之作用很可能是由於其增加抗氧化酵素活性與降低脂質過氧化作用所造成。這一部份之結果已發表在 J Plant Physiol 期刊，有興趣之學者可逕行參攷。

水稻葉片經脫水(DH)與 PEG 處理可加速老化（圖 1）。NO 釋放劑可克服脫水與 PEG 所加速之老化，而 c-PTIO 可使 NO 釋放劑之作用消失(圖 1、表 1 與表 2)。Sorbitol (ST)是一種滲透劑，它亦可造成水稻葉片老化之加速，但 NO 釋放劑卻不能克服 ST 所造成之老化之加速（表 3）。ST 處理不會造成氧化逆境，然而脫水與 PEG 處理會造成水稻葉片氧化逆境（表 4）。換言之，NO 釋放劑所以會克服脫水與 PEG 所造成之老化加速，係由其會造成氧化逆境；NO 釋放劑不能克服 ST 所造成老化之加速，是由於 ST 不會造成氧化逆境。NO 釋放劑可抑制脫水與 PEG 處理所降低之 superoxide dismutase 活性及所增加之脂質過氧化作用（表 4）。

ABA、MJ 與過量鎘均會造成水稻葉片氧化逆境，而加速葉片之老化。試驗結果也顯示，NO 釋放劑可經由其改變抗氧化酵素活性與降低脂質過氧化作用，進而克服 ABA、MJ 與過量鎘所加速之葉片老化。

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 NO 與水稻葉片氧化逆境間之關係有明確之瞭解

五、參考文獻

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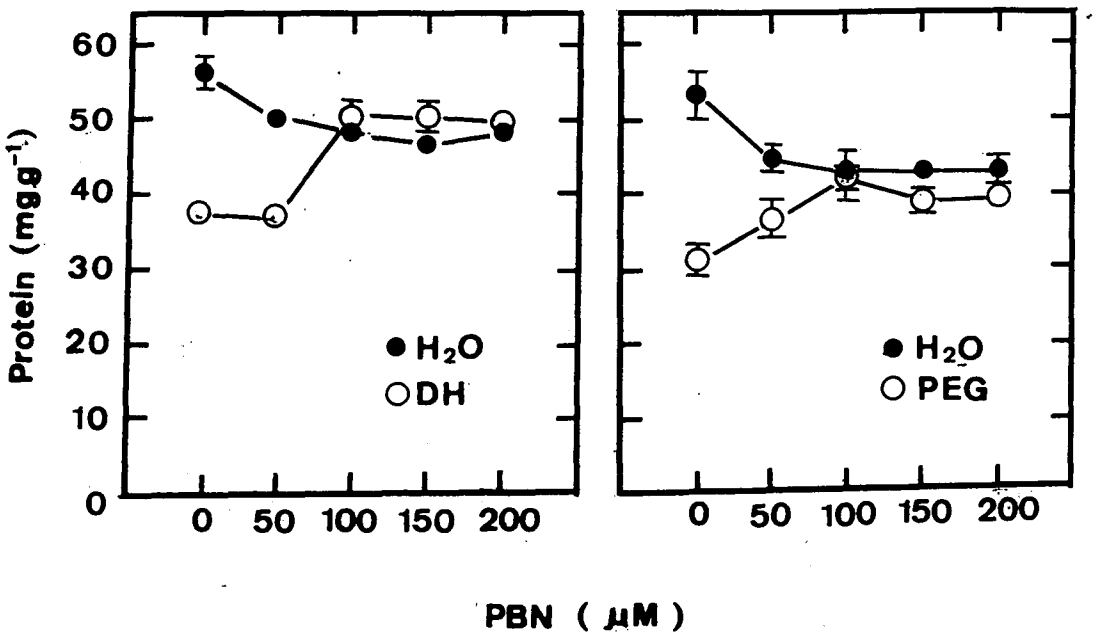

Figure 1. Effect of PBN concentrations on protein contents in rice leaves treated with either distilled water, dehydration (DH) or polyethylene glycol (PEG). For PEG experiment, protein was determined 12 h after treatment in the light. For DH experiment, detached rice leaves were pretreated with distilled water on various concentrations of PBN for 6 h in the light and then were treated with distilled water or subjected to dehydration for 12 h. Vertical bars represent standard errors (n = 4). Only those standard errors larger than the symbol are shown.

Table 1. Effect of NO donors on protein content in dehydrated rice leaves in the presence or absence of c-PTIO

Treatment	Protein, mg g^{-1}
H_2O	59.8 ±1.2
DH	47.6 ± 2.3
DH + PBN	55.8 ± 1.2
DH + SIN-1	53.2 ± 0.5
DH + SNP	55.2 ± 0.7
DH + Asc + $NaNO_2$	53.2 ± 0.5
DH + PBN + c-PTIO	49.6 ± 0.6
DH + SIN-1 + c-PTIO	48.1 ± 0.6
DH + SNP+ c-PTIO	48.3 ± 2.6
DH + Asc + $NaNO_2$ + c-PTIO	48.5 ± 3.0

Detached rice leaves were pretreated with distilled water or NO donors in the presence or absence of c-PTIO for 6 h in the light and then treated with distilled water and subjected to dehydration (DH) for 12 h. The concentrations of PBN, SIN-1, SNP, Asc, $NaNO_2$, and c-PTIO were 100, 100, 100, 100, 200, and 100 μM, respectively. Data are the means ± standard errors (n = 4).

Table 2. Effect of NO donors on protein content in PEG-treated rice leaves in the presence or absence of c-PTIO

Treatment	Protein, mg g^{-1}
H_2O	56.8 ± 0.5
PEG	47.2 ± 1.7
PEG + PBN	51.1 ± 1.6
PEG + SIN-1	51.7 ± 1.4
PEG + SNP	52.0 ± 1.6
PEG + Asc + $NaNO_2$	51.9 ± 1.5
PEG + PBN + c-PTIO	46.4 ± 0.7
PEG + SIN-1 + c-PTIO	46.2 ± 1.7
PEG + SNP+ c-PTIO	46.0 ± 0.4
PEG + Asc + $NaNO_2$ + c-PTIO	47.7 ± 1.0

The concentrations of PBN, SIN-1, SNP, Asc, $NaNO_2$, and c-PTIO were 100, 100, 100, 100, 200, and 100 μM, respectively. Protein content was determined 12 h after treatment in the light, data are the means ± standard errors (n = 4).

Table 3. Effect of NO donors on protein content in sorbitol (ST)-treated rice leaves in the presence or absence of c-PTIO

Treatment	Protein, mg g^{-1}
H_2O	55.2 ± 0.6
ST	49.8 ± 0.9
ST + PBN	50.0 ± 1.6
ST + SIN-1	49.4 ± 3.3
ST + SNP	47.1 ± 1.7
ST + Asc + $NaNO_2$	48.8 ± 1.4

The concentrations of PBN, SIN-1, SNP, Asc, and $NaNO_2$ were 100, 100, 100, 100, and 200 μM, respectively. Protein content was determined 12 h after treatment in the light, data are the means ± standard errors (n = 4).

Table 4. Effect of PBN on MDA content and SOD activity in PEG-, ST-treated and dehydrated rice leaves in the presence or absence of c-PTIO

Treatment	MDA, nmol g^{-1}	SOD, units mg^{-1} protein
H_2O	40.3 ± 0.9	1.70 ± 0.03
ST	43.4 ± 2.1	1.51 ± 0.09
PEG	67.3 ± 1.6	1.04 ± 0.14
PEG + PBN	53.1 ± 1.4	1.59 ± 0.10
PEG + PBN + c-PTIO	68.2 ± 2.1	0.99 ± 0.14
H_2O	40.3 ± 0.4	1.70 ± 0.04
DH	93.4 ± 1.2	0.73 ± 0.02
DH + PBN	76.5 ± 2.3	1.17 ± 0.03
DH + PBN + c-PTIO	91.1 ± 1.5	0.71 ± 0.01

The concentrations of PBN and c-PTIO were 100 and 100 μM, respectively. For sorbitol (ST) and polyethylene glycol (PEG) experiments, MDA and SOD were determined at 12 h after treatment in the light. For dehydration (DH) experiment, detached rice leaves were pretreated with distilled water or PBN in the presence or absence of c-PTIO for 6 h in the light and then treated with distilled water or subjected to dehydration for 12 h. Data are the means ± standard errors (n = 4).