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Presoaking with nitric oxide donor SNP alleviates heat shock damages in mung bean leaf discs

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ABSTRACT. The objective of this study was to examine whether exogenously applied nitric oxide (NO) have some protective role on mung bean (*Phaseolus radiatus*) leaf discs under heat shock. Leaf discs were presoaked in distilled water and sodium nitroprusside (SNP, a NO donor) solution (150 μM) for 60 min respectively, then submitted to a heat shock at 45°C for 90 min in dark. Control materials were presoaked with distilled water and laid under room temperature (25°C). The chlorophyll *a* fluorescence parameters, membrane integrity, hydrogen peroxide (H_2O_2) content and activities of antioxidant enzymes [catalase (CAT), guaiacol peroxidase (POD) and superoxide dismutase (SOD)] were assayed. Compared with heat-shocked leaf discs, the maximal quantum yield of photosystem II (PSII) (measured as F_v/F_m) was significantly increased, electrolyte leakage due to heat shock was reduced by 48%, lipid peroxidation and H_2O_2 content were kept at control level by SNP presoaking. The suppressed activities of antioxidant enzymes by heat shock were all recuperated by SNP presoaking. On the other hand, these role of SNP presoaking were reversed fully or partially by bovine hemoglobin (a powerful NO scavenger), suggesting that protective effect by SNP is attributable to released NO. In conclusion, it appears that the exogenously applied NO donor SNP can promote higher leaf photochemical activity and cell membrane integrity in mung bean leaf discs under heat shock. This role is putatively due to that the released NO can recuperate suppressed activities of anti-oxidant enzymes, thus eliminating oxidative damage under heat shock stress.

Keywords: Antioxidant enzyme activity; Heat shock; Membrane integrity; Mung bean (*Phaseolus radiatus*); Nitric oxide; Photochemical efficiency.

INTRODUCTION

Heat stress can influence many physiological processes or factors in plants, e.g., inhibition of photosynthesis, limitation of carbohydrate accumulation and destruction of cell membranes and cytoskeleton (Liu and Huang, 2000). Usually, the primary site of damage associated with non-optimal temperatures was indicated to be the photosynthetic apparatus (Yamane et al., 1998; Bukhov et al., 1999), and PSII (the H_2O-oxidising, quinine-reducing complex) was the most heat sensitive of the chloroplast thylakoid-membrane protein complexes involved in photosynthetic electron transfer and ATP synthesis (Heckathorn et al., 1998). The adverse effects of heat stress may be related to the overproduction of reactive oxygen species (ROS) (i.e. superoxide anion [$^{\bullet}O^{2-}$], hydrogen peroxide [H_2O_2], hydroxyl radical [$^{\bullet}OH$] and singlet oxygen [$^1O_2$]) (Pastori and Foyer, 2002).

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Plants have well-developed enzymatic and nonenzymatic scavenging systems to quench ROS (Vranová et al., 2002). So the ROS is always generated at a controlled balance under unstressed conditions. When plants are subjected to adverse conditions, the scavenging system may lose its function and the balance between producing and quenching ROS can be disturbed, resulting in accumulation of ROS, which in general, cause lipid peroxidation, protein modification, breakage of DNA strands, chlorophyll decay, ion leakage and cell death (Scandalios, 1993).

Nitric oxide (NO) is a bioactive molecule involved in many biological events, and its effects have been reported either protective or toxic in plants (Beligni and Lamattina, 2001). It can act as a signal molecule in plant defense interactions with microorganisms (Dangl, 1998); or as a compound with hormonal properties (Leshem and Haramaty, 1996) to affect photomorphogenesis (Beligni and Lamattina, 2000), and to play a central role in determining lateral root development (Correa-Aragunde et al., 2004). At the same time, NO may act as an antioxidant to reduce toxicity induced by herbicide paraquat, heavy metal or H_2O_2 and to delay the senescence of rice leaf induced

by methyl jasmonate or abscisic acid (Hung et al., 2002; Hung and Kao, 2003, 2004, 2005; Hsu and Kao, 2004; Laspina et al., 2005). However, mechanical stresses, such as centrifugation, induced *Arabidopsis* to produce NO, which further cause DNA fragmentation (Garcés et al., 2001).

The exogenous application of NO donors conferred an increased tolerance to severe drought stress conditions in plants, with higher water retention, less transpiration rate, higher proportion of stomatal closure and so (García Mata and Lamattina, 2001). Uchida et al. (2002) reported that NO can increase both salt and heat tolerance in rice seedlings by sodium nitroprusside (SNP, 1–10 μM) in the hydroponic solution for 2 days. Considering that the temperature of an individual plant cell can change in the time range of a few minutes to hours (Pastenes and Horton, 1996), being much more rapid than other factors that cause stress (e.g. water levels or salt levels) (Larkindale and Knight, 2002), this study aimed to further explore the protective role of exogenously applied NO in plant tissues submitted to a rapid heat stress.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of mung bean (*Phaseolus radiatus*) were germinated on moist paper towels and transferred in plastic pots containing a sterile mixture of soil: vermiculite (3:1, v/v). Plants were grown in a greenhouse exposed to direct full sunlight, watered daily and fertilized with a complete nutrient medium every three days. The fully-expanded healthy leaflets (on different compound leaves) were excised during mid-morning (about two hours after sunrise), midribs removed and cut into discs with a diameter of 1.5 cm. Leaf discs were immersed in small beakers containing distilled water, or 150 μM SNP (an NO donor), or SNP (150 μM) plus bovine hemoglobin [4 g L^{-1}, a powerful NO scavenger (Takahashi and Yamasaki, 2002)]. After submitted to a pulse of 45 s of vacuum, the beakers were left 1 h in a plant incubator with a temperature of 25°C and a photosynthetic photon flux density (PPFD) of about 20 μmol m^{-2} s^{-1}. Referred to the method of rapid heat stress (Law and Crafts-Brandner, 1999), presoaked leaf discs were put on moist filter papers in Petri dishes and laid in a plant incubator at a prearranged temperature 45°C with no light illumination for 90 min. One Petri dish containing water-pretreated leaf discs was left at 25°C in dark as the control. So there were four treatments in the present experiment, i.e. 25°C with water presoaking (Control), 45°C with water presoaking (H), 45°C with SNP presoaking (S), 45°C with SNP and Hb presoaking (S+Hb).

Chlorophyll fluorescence measurement

The initial fluorescence (F_o), maximal fluorescence (F_m) in leaf discs after treatments were determined using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd., UK) in the dark room. The saturating PPFD of the actinic light was 3000 μmol m^{-2} s^{-1} (0.8 s). The ratio of F_v/F_m, where the variable fluorescence yield, F_v, is defined as (F_m-F_o), is a direct measure of the maximal quantum yield of PSII (Meyer and Santarius, 1998).

Electrolyte leakage and TBARS contents

Cell membrane permeability was measured via electrolyte leakage (Meyer and Santarius, 1998). Treated leaf discs were washed in deionised water to remove surface ions and fully immersed in 50-ml-glass bottles containing 25 ml of distilled water of known conductivity. The bottles were capped and shaken in plant incubator at 25°C without light illumination for 12 h. Then the conductance was measured using an Multiline P4 Universal Meter (WTW, Weilheim, Germany). All electrolyte leakage data were expressed as conductivity readings related to the material mass after treatments, i.e., unit was μS cm^{-1} g^{-1}.

The extent of lipid peroxidation in leaf discs was determined in terms of thiobarbituric acid-reacting substances (TBARS) content by the method of Fryer et al. (1998).

H_2O_2 Content

According to the method of Freguson et al. (1983) with some modification, treated leaf discs (about fresh weight 1.5 g) were homogenized quickly in 3 ml of cold acetone with a mortar and pestle in an ice bath, centrifuged at 5,000 *g* for 10 min at 4°C. The supernatant (0.2 ml) was complement to 1.0 ml with 0.8 ml cold acetone, and fully mixed with 0.1 ml of 20 % $TiCl_4$-HCl solution and 0.2 ml strong ammonia hydroxide, centrifuged at 3,000 *g* for 10 min. The resulted peroxide-Ti compound was washed with acetone for 3~5 times and dissolved in 3 ml 2 M H_2SO_4 and the absorbance was measured at 410 nm. The content of H_2O_2 (μmol g^{-1} FW) was calculated by comparison with a standard curve relating H_2O_2 concentrations to absorbance.

Enzymes activities

Treated leaf discs (about 0.3~0.4 g) were homogenized in 2.5 ml cold extraction buffer [50 mM phosphate buffer (pH 7.0), containing 1% (m/v) polyvinylpyrrolidone] with a mortar and pestle in an ice bath, centrifuged at 15000 *g* for 20 min at 4°C. The supernatant was used for assays of catalase (CAT; EC 1.11.1.6) and guaiacol peroxidase (POD; EC 1.11.1.7) activity. The same process was conducted for extracting superoxide dismutase (SOD; EC 1.15.1.1) except that the extraction buffer was 50 mM phosphate buffer (pH 7.8) [containing 2% (m/v) polyvinylpyrrolidone, 0.3% (v/v) triton X-100, and 0.1 mM EDTA].

Activities of CAT and POD were measured according to Liu and Huang (2000) with some modification. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2, and 0.05 ~ 0.1 ml enzyme extract. Reaction was initiated by adding enzyme extract. The absorbance change at 240 nm per min was recorded and calculated automatically. The POD reaction solution

(3 ml) contained 100 mM phosphate buffer (pH 6.0), 20 mM guaiacol, 40 mM H_2O_2, and 0.1 ml enzyme extract. Absorbance changes at 470 nm were monitored as in CAT activity measurement. One unit CAT or POD activity was defined as an absorbance change of 0.01 per min.

Exactly according to the method of Cavalcanti et al. (2004), the activity of SOD was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction. Considering that there are three SOD isoenzymes with different sensibility to KCN and H_2O_2 (Alscher et al., 2002), enzyme extracts were mixed respectively with the equal volume of KCN (1.5 mM) or H_2O_2 (1 mM), and incubated at 25°C for 10 min. The residual activities of these treated enzyme extracts were determined to calculate the activity of different isoenzymes.

All of the above enzyme activities were measured by using UV-1601 UV-Visible Spectrophotometer (Shimadzu Corporation, Japan).

RESULTS

Chlorophyll fluorescence parameter

As shown in Table 1, heat shock caused a significant decrease about 50% to the maximal quantum yield of PSII (F_v/F_m), while SNP presoaking could alleviate this negative effect about 16.5%, both relative to control. Presoaking with SNP + bovine hemoglobin (Hb) blocked the action of SNP. The initial fluorescence (F_o) in four treatments had no statistical difference, meaning that heat shock and SNP presoaking to influence F_v/F_m were mainly through their action on the maximal fluorescence (F_m) (Table 1). This suggested that exogenous NO could improve the electron flux from the primary quinine acceptor (Q_A) to the electron transfer chain (Ortiz and Cardemil, 2001).

Considering that besides NO, NO_2^- and $[Fe(CN)_6]^{3-}$ are also products when SNP dissolved in water (Ruan et al., 2004), $NaNO_2$ and $K_3[Fe(CN)_6]$ as well as 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, another NO scavenger) were used to test if the role of SNP presoaking was solely due to NO released (Table 2). After heat shock, SNP presoaking significantly increased F_v/F_m compared with H_2O presoaking, while F_v/F_m values had no statistical difference in discs presoaked with H_2O, SNP plus NO scavengers (Hb or cPTIO) and byproducts of SNP dissolution [NO_2^-, $Fe(CN)_6^{3-}$]. This result further confirmed that the role of SNP presoaking was attributed to released NO, not other derived compounds.

Membrane permeability, lipid peroxidation and H_2O_2 content

The degree of cell membrane injury induced by stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji et al., 2002). As shown

Table 1. Chlorophyll *a* fluorescence parameters in treated leaf discs. Values in brackets are percentage of control. Leaf discs were treated as described in "Materials and Methods".

Parameters	Treatments			
	Control	H	S	S + Hb
F_o	658 ± 6 a	656 ± 6 a (99.7%)	652 ± 2 a (99.1%)	653 ± 5 a (99.2%)
F_m	4071 ± 15 a	1138 ± 53 b (28.0%)	1486 ± 67 c (36.5%)	1159 ± 47 b (28.5%)
F_v/F_m	0.838 ± 0.001 a	0.421 ± 0.030 b (50.2%)	0.559 ± 0.018 c (66.7%)	0.433 ± 0.026 b (51.7%)

Control, 25°C incubation with water presoaking; H, 45°C heat shock with water presoaking; S, 45°C heat shock with 150 μM SNP presoaking; S + Hb, 45°C heat shock with presoaking of 150 μM SNP plus 4 g L^{-1} bovine hemoglobin. In each row values followed by the same letter are not significantly different at $P<0.05$ (n = 8).

Table 2. Values of F_v/F_m in heat shocked leaf discs presoaked with different solutions. Leaf discs were treated as described in "Materials and Methods".

Treatments	Presoaked respectively in following solutions before 45°C heat shock					
	H_2O	SNP	SNP + Hb	SNP + cPTIO	$NaNO_2$	$K_3[Fe(CN)_6]$
F_v/F_m	0.423 ± 0.034 a	0.561 ± 0.022 b	0.431 ± 0.031 a	0.429 ± 0.017 a	0.426 ± 0.013 a	0.418 ± 0.024 a

These different solutions were H_2O, SNP (150 μM), SNP (150 μM) + Hb (4 g L^{-1}), SNP (150 μM) + cPTIO (150 μM), $NaNO_2$ (150 μM), $K_3[Fe(CN)_6]$ (150 μM). Values of F_v/F_m followed by the same letter are not significantly different at $P<0.05$ (n = 6).

in Figure 1, heat shock increased the electrolyte leakage by 105% in water-presoaked leaf discs, by 57% in SNP-presoaked and by 81% in SNP + Hb presoaked ones. This meant that SNP presoaking would decrease the electrolyte leakage by 48%, while NO scavenger Hb could reverse the effect of SNP presoaking by 24% (Figure 1).

TBARS are the product of lipid peroxidation, and higher levels of these substances are found in plants that are subject to higher levels of oxidative stress (Larkindale and Knight, 2002). As shown in Figure 1, after heat shock, TBARS contents in leaf discs with water presoaking or SNP+Hb presoaking were significantly higher than in control, while there was no statistical difference between SNP-presoaked leaf discs and control. This suggested that SNP presoaking would reduce the lipid peroxidation degree in heat-shocked tissue to the non-heat shocked level and this role was due to released NO.

A previously suggested role of NO as antioxidants to scavenge ROS was tested by directly measuring the H_2O_2 content in present work (Figure 1). Compared with control discs, H_2O_2 content in SNP-presoaked leaf discs had no statistical difference after heat shock, while H_2O_2 content in both water presoaked and SNP+Hb presoaked discs were significantly higher than control. This suggested that heat shock would result in H_2O_2 production while NO would significantly decrease heat shock-induced H_2O_2 to the control level.

Activities of antioxidant enzymes

The activities of radical scavenger antioxidant enzymes (CAT, POD and SOD) were declined significantly in water presoaked leaf discs by heat shock. SNP presoaking would increase POD activity by 16% and recuperate the suppressed activities of CAT and SOD to the control level. However, NO scavenger Hb could fully or partially reverse these influences (Figure 2).

Activities of SOD isoenzymes

The three isoforms of SOD [i.e. iron SOD (Fe SOD), manganese SOD (Mn SOD) and copper-zinc SOD (Cu-Zn SOD)] are located in different compartments of the cell, and exhibit different responses to the same kind stress (see Kliebenstein et al., 1998; Alscher et al., 2002). In the present research, Mn SOD activity remained approximately constant in four treatments. Activities of Cu-Zn SOD and Fe SOD were significantly decreased by heat shock in leaf discs with water presoaking or SNP+Hb presoaking, while were recuperated by SNP presoaking (Figure 3).

DISCUSSION

PSII photochemical activity

Environmental stresses that affect PSII efficiency lead to a characteristic decrease in the maximal quantum yield of PSII (measured as F_v/F_m) (Krause and Weis, 1991). So F_v/F_m was employed to compare the heat stress tolerance between different plants (Ortiz and Cardemil, 2001; Liu and Huang, 2000; Law and Crafts-Brandner, 1999), and to estimate the role of exogenously applied chemicals on plant tissue submitted to heat stress (Logan and Monson, 1999), referring to that a higher F_v/F_m after stress stands for a higher thermotolerance, or higher protective role of the applied chemical. In the present work, the significantly increased F_v/F_m due to exogenous NO, relative to water

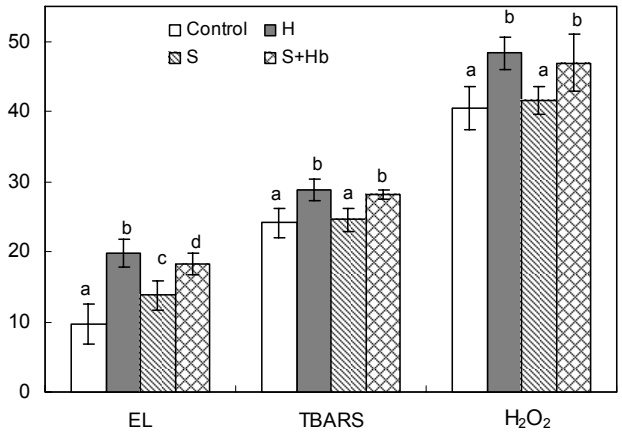

Figure 1. Electrolyte leakage (EL), lipid peroxidation level (TBARS contents) and H_2O_2 content in four treatments. Control, 25°C incubation with water presoaking; H, 45°C heat shock with water presoaking; S, 45°C heat shock with 150 μM SNP presoaking; S + Hb, 45°C heat shock with presoaking of 150 μM SNP plus 4 g L^{-1} bovine hemoglobin. The unit for electrolyte leakage (EL) is μS cm^{-1} g^{-1} FW; for TBARS content is nmol g^{-1} FW; for H_2O_2 content is μmol g^{-1} FW. Bars, SE (n = 6).

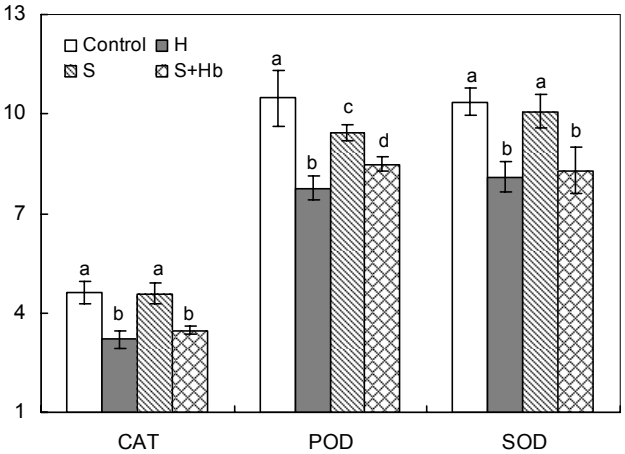

Figure 2. The activities of catalase (CAT), guaiacol peroxidase (POD) and superoxide dismutase (SOD) in four treatments. Control, 25°C incubation with water presoaking; H, 45°C heat shock with water presoaking; S, 45°C heat shock with 150 μM SNP presoaking; S + Hb, 45°C heat shock with presoaking of 150 μM SNP plus 4 g L^{-1} bovine hemoglobin. Calculation of enzyme activity was described in "Materials and methods". The activity unit for CAT and POD is 10^3 U min^{-1} g^{-1} FW; for SOD is U min^{-1} g^{-1} FW. Bars, SE (n = 6).

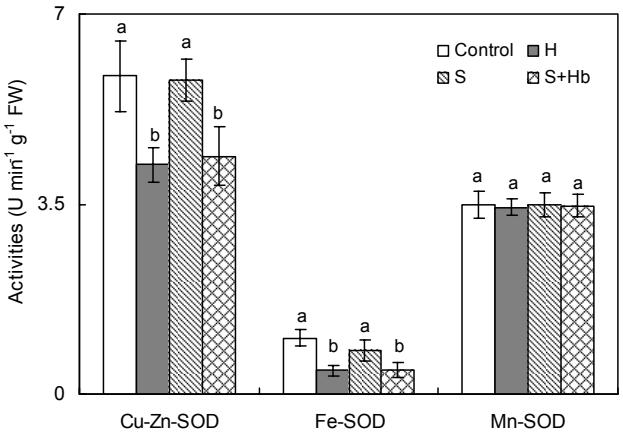

Figure 3. The activity of superoxide dismutase (SOD) isoenzymes (i.e. Cu-Zn-SOD, Fe-SOD, and Mn-SOD) in four different treatments. Control, 25°C incubation with water presoaking; H, 45°C heat shock with water presoaking; S, 45°C heat shock with 150 μM SNP presoaking; S + Hb, 45°C heat shock with presoaking of 150 μM SNP plus 4 g L^{-1} bovine hemoglobin. Bars, SE (n = 3).

presoaking or SNP+Hb presoking, meant an evident protective role on leaf discs under heat stress (Table 1). Considering that decrease in F_o was suggested to be associated with an increased capacity for energy dissipation within light-harvesting complexes (Demmig-Adams, 1990), and increase in F_o to be related to partly reversible inactivation or irreversible damage in the reaction centers of PSII (Yamane et al., 1997), the statistically similar F_o in four treatments (Table 1) meant that PSII reaction centers was not influenced by heat shock or SNP presoaking in dark.

Cell membrane integrity

Cell membranes are one target of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under stress conditions is a major component of stress tolerance in plants (Bajji et al., 2002). Lipid peroxidation is commonly taken as an indicator of oxidative stress and is quantified by the thiobarbituric acid (TBA) test, which is easy to perform and allows the results to be conveniently expressed as TBARS (Iturbe-Ormaetxe et al., 1998). However, results from the TBA test need to be compared with more specific assays, because the hydroxyl radical ($^{\bullet}OH$) and other highly reactive radical species can oxidize proteins in addition to lipids (Iturbe-Ormaetxe et al., 1998). In the present study, besides TBARS contents, membrane integrity was also assayed by directly measuring the electrolyte leakage from plant tissue because electrolyte leakage from the cell is regarded as a consequence of an oxidative burst leading to membrane peroxidation (Bajji et al., 2002). Although the TBARS content was alleviated to the control level by exogenous NO, the electrolyte leakage was still significantly higher than that in control despite of NO action (Figure 1). This meant exogenous NO could reduce the lipid peroxidation degree in heat-shocked tissue to the control level, but could not recuperate the membrane integrity fully. The similar result was reported in wheat plants submitted to drought and UV-B irradiation in combination (Alexieva et al., 2001). It was suggested that heat shock may destroy the cell membrane integrity through some way besides lipid peroxidation. The possible one was the oxidative denaturation of membrane protein, and exogenous NO could not prevent this process.

Antioxidant enzyme activity

After heat shock, activities of antioxidant enzymes (CAT, POD and SOD) decreased in water presoaked leaf discs and partially or fully recuperated due to SNP presoaking (Figure 2). Because the physiological role of CAT and POD is to break down H_2O_2 in the cell, decreases in activities of these two enzymes would result in H_2O_2 accumulation (Figure 1). SOD is the key enzyme to catalyze the conversion of $^{\bullet}O_2^-$ into H_2O_2 and O_2, and reduction in SOD activity would be related to accumulation of $^{\bullet}O_2^-$. Through Herbert-Weiss reaction, H_2O_2 and $^{\bullet}O_2^-$ react and form the most reactive hydroxyl radical ($^{\bullet}OH$), which can directly attack unsaturated fatty acids of lipid to induce lipid peroxidation in the cell (Bowler et al., 1992). The decreases in activities of CAT, POD and SOD suggested that the ROS-scavenging ability was partially destroyed by heat shock. SNP presoaking recuperated the antioxidant enzyme activities in heat-shocked leaf discs, which may eliminate the possible accumulation of ROS and reduce the oxidative damage induced by heat shock (Figure 1). Therefore, it was suggested putatively that the role of exogenous NO may act partially through its capacity to increase the antioxidant enzyme activities.

SOD is unique in that its activity controls the concentrations of $^{\bullet}O_2^-$ and H_2O_2 and therefore likely to be central in the defense mechanism (Bowler et al., 1992). In this study, after heat shock, activities of Cu-Zn SOD and Fe SOD decreased in water presoaked leaf discs and was recuperated by exogenous NO (Figure 3). Since Cu-Zn SOD and Fe SOD both were thermostable in present experimental conditions (see Asada, 1999), the decrease in their activity was not caused by enzyme protein denaturation in heat shock. However, the inactivation of H_2O_2 scavenging enzymes (CAT and POD) by heat shock would result in H_2O_2 accumulation in leaf discs with water presoaking or SNP+Hb presoaking (Figure 1), thus decreasing the activities of Cu-Zn SOD and Fe SOD due to their susceptibility to H_2O_2 (Asada, 1999). Therefore, it is indicated that the recuperation of Cu-Zn SOD and Fe SOD activities should be related to the improved activities of CAT and POD by exogenous NO (Figure 2). On the other hand, the activity of SOD can be induced by diverse stress conditions and the effect of a particular stress on SOD gene expression is likely to be governed by the subcellular sites at which oxidative stress is generated (Bowler et al., 1992). For example, in *N. plumbginifolia*, mitochondrial Mn SOD responds to increased oxyradical formation in the mitochondria while chloroplastic Fe SOD responds to such an event occurring in the chloroplasts (Tsang et

al., 1991). Cytosolic Cu-Zn SOD probably responds to cytosol-localized reactions in a similar fashion (Bowler et al., 1992). So, that Mn SOD activity remained unchanged in the four treatments (Figure 3) may putatively indicate that chloroplast and cytosol were the primary targets of heat shock under present experimental conditions.

In general, the presented data here show that exogenous NO could significantly alleviate the negative effects of a heat shock at 45°C for 90 min on photochemical activity of PSII, cell membrane integrity, and antioxidant enzyme activities, thus improving the performance of mung bean leaf discs under heat shock.

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用一氧化氮釋放劑 SNP 預浸泡可緩解熱激對綠豆葉圓片的傷害

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本研究主要是探討外源施用的一氧化氮（NO）是否對熱激脅迫下的綠豆（*Phaseolus radiatus*）葉圓片具有一定的保護作用。綠豆葉圓片在蒸餾水和 150 μM 的一氧化氮釋放劑 SNP 中分別預浸泡 60 分鐘，然後在 45ºC 黑暗下熱激處理 90 分鐘。對照材料用蒸餾水預浸泡並放置在室溫（25ºC）。以葉綠素螢光參數、細胞膜完整性、過氧化氫含量和抗氧化酶 [catalase (CAT), guaiacol peroxidase (POD) 與 superoxide dismutase (SOD)] 的活性為考察指標。與蒸餾水預浸泡-熱激處理的葉圓片相比較，SNP 預浸泡可顯著提高光系統 II 的最大量子產率（F_v/F_m）、使熱激導致的電解質滲漏減少 48%、使膜脂過氧化程度和過氧化氫含量保持在對照水準、因熱激而受抑的抗氧化酶活性也因 SNP 預浸泡而不同程度地恢復。另一方面，SNP 預浸泡的這些作用能被牛血紅蛋白（一種有效的 NO 清除劑）所完全或部分去除，表明 SNP 的保護性作用應主要歸因於所釋放的 NO。本實驗顯示，外源施用的 NO 釋放劑 SNP 能提高綠豆葉圓片在熱激下的光化學活性和細胞膜完整性，該作用可能是由於所釋放的 NO 恢復了熱激下受抑的抗氧化酶的活性，從而減輕了氧化傷害。

關鍵詞：抗氧化酶活性；熱激；膜完整性；綠豆；一氧化氮；光化學活性。