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RESEARCH ARTICLE

Modulation by S-nitrosoglutathione (a natural nitric oxide donor) of photosystem in *Pisum sativum* leaves, as revealed by chlorophyll fluorescence: Light-dependent aggravation of nitric oxide effects

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Abstract

The reported effects of nitric oxide (NO), a signaling molecule, on the photochemical components of leaves are ambiguous. We examined the changes by a natural NO donor, S-nitrosoglutathione (GSNO). The effect of GSNO on *Pisum sativum* leaves was studied after a 3-hour exposure in dark, moderate (ML), or high light (HL). The NO levels in GSNO-treated samples were at their maximum under HL, compared to those under ML or dark. Most of the elevated NO was decreased by 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), a NO scavenger, confirming the NO increase. Treatment with GSNO caused inhibition of photosynthesis/respiration and restricted electron transport mediated by both photosystem (PS)II and PSI. However, the inhibition by NO-donor of PSII components was stronger than those of PSI. A marked increase in the PSI acceptor side limitation [Y(NA)] and a decrease in PSI donor side limitation [Y(ND)] indicated an upregulation of cyclic electron transport, possibly to balance the damage to PSII by GSNO. We suggest that NO aggravated the HL-induced inhibition of photosynthesis and dark respiration.

Keywords

Chl fluorescence; nitric oxide; photosynthesis; photosystems; respiration; high light.

Introduction

Abiotic stress affects photosynthesis in higher plants by targeting the photosynthetic apparatus, particularly photosystem (PS) II (1, 2). A common consequence of abiotic/biotic stress is the increase in both reactive oxygen species (ROS) and nitric oxide (NO) levels in plant cells (3, 4). ROS and NO could exert multiple effects on photosynthetic and mitochondrial metabolisms (5, 6). The impact of ROS on photosynthesis was studied by several groups (7, 8), while the studies with NO are just catching up (9). Nitric oxide (NO), can modulate various physiological processes in plants (9, 10). There are contrasting claims that NO can be harmful or beneficial for plant cells against stress (4, 11, 12).

In addition to its role as a primary signal, NO may affect respiration and photosynthesis. However, the reports on the modulation by NO of photosynthesis, particularly photosystems (PSI and PSII) are ambiguous. Studies on chlorophyll (Chl) fluorescence indicated that the PSII-related reactions were either decreased (13) or increased on exposure to NO (14). Some studies have suggested that NO may protect PSII components from osmotic or salinity stress (15, 16). Most of these studies were with sodium nitroprusside

(SNP). The variation in the reports appeared to be due to different NO-donors (17) or different instruments used for monitoring Chl fluorescence. Most of the experiments were done with leaves, e.g., pea (17) and potato (18), while some were with protoplasts of pea (6, 19).

We chose pea (*Pisum sativum*), which was easy to grow in a typical greenhouse. Within 12-14 days, pea leaves were ready. Due to their thin nature, treating pea leaves with test chemicals is quite effective. In our laboratory, several experimental protocols were standardized with pea leaves. For most of the experiments, we used leaf discs, but for the PSII and PSI measurements, we used whole detached leaves.

We reexamined the modulation of photosynthesis and PSII/PSI components in pea leaves by a natural NO donor, S-nitrosoglutathione (GSNO). The leaf discs were treated with NO donor under varied conditions: dark, moderate light (ML), or highlight (HL). Chlorophyll fluorescence induction patterns were used for assessing the status of PSII and PSI.

Materials and Methods

Plant material and growth conditions

We described earlier (20), the germination of pea (*Pisum sativum* L., cv. Arkel) plants from seeds and their growth. The leaf discs (28 equivalent to 100 mg) were kept for 3 h in dark or moderate (ML, 300 µE $m^{-2} s^{-1}$) or high-light (HL, 1200 µE $m^{-2} s^{-1}$). When required, other test compounds were included at the final concentrations mentioned.

Quantification of NO content

The NO levels in leaf discs were determined by Griess Reagent (21). Briefly, after the treatment, the leaf discs (400 mg) were ground in 3 ml of 50 mM chilled acetic acid buffer (pH 3.6). The extracts were centrifuged (10,000 g for 15 min at 4 °C) and the supernatant was collected. The pellet was rinsed twice with 1 ml of extraction buffer and centrifuged again. The supernatants were combined, and 100 mg of charcoal was added. The filtrate was vortexed, cleared by a syringe filter, and collected into a new tube. One ml of Griess Reagent was added to 1 ml of filtrate, and the absorbance was determined at 540 nm, after 30 min. Griess Reagent indicates NO levels but may not be perfect for estimating NO. We used cPTIO, a scavenger of NO, to validate the NO levels. The decrease in the values in the presence of NO scavenger can be taken as valid values of NO content.

Measurement of Photosynthesis and Respiration

Photosynthesis and respiration rates at 25°C were measured by a leaf disc oxygen electrode system (Hansatech Instruments, UK). Light-emitting diodes provided the light. 200 µl of 1 M bicarbonate buffer (pH 9.0) moistened the uppermost capillary matting, creating 5% (v/v) CO_2 in the chamber. Three rings of one, six, and twelve-leaf discs were symmetrically arranged on this matting. The oxygen level in the chamber was calibrated each time for the sample (22).

Determination of PSII and PSI efficiency

Dual-PAM 100 (Walz, Germany) was used to assess the efficiency of PSII and PSI components. Pre-dark-adapted leaves were treated as required and were kept in darkness for 30 minutes. The PSII and PSI-based Chl fluorescence parameters were measured at 800 and 1000 PAR (µE $m^{-2} s^{-1}$), respectively (23, 24). The PSII parameters were calculated as follows: Maximum photosynthetic efficiency: $Fv/Fm = (Fm - Fo)/Fm$; Quantum yield of PSII: Y(II) = $(Fm'-Ft)/Fm'$; Non-regulated energy dissipation: Y(NO) = Ft/FM; Yield of regulatory photo-protective energy dissipation: Y(NPQ) = $(Fm' - Ft/Fm)/Ft$; non-photochemical quenching: NPQ, qP, and qL.

The following PSI parameters were calculated as described (23, 24). Maximum photosynthetic efficiency of PSI (Pm); Yield of PSI: Y(I) = $(Pm' - P)/Pm$; PSI acceptor side limitation: Y(NA) = $Pm - Pm')/Pm$; and PSI donor side limitation: Y(ND) = P/Pm. Further details are in the legends of Figure 3 and Figure 4. The manufacturer manual has additional information.

Replications and statistical analysis

The presented results are averages ± SE of at least three independent experiments conducted on different days. ANOVA checked the significance. $*P < 0.05$; $**P < 0.001$.

Results

The present work focused on the modulation by GSNO of the PSI/PSII components in pea leaves based on Chl fluorescence. Initially, we ascertained the leaf NO content and checked photosynthesis/respiration rates.

Increase in NO content on treatment with NO donor

The effects of GSNO were checked at different concentrations. Maximum release of NO occurred in the presence of 1 mM GSNO. The extent of increase in NO by GSNO in dark or ML or HL ranged from 7 to 11-fold. The NO contents were markedly dampened in the presence of cPTIO, a NO scavenger (Figure 1), indicating that the detected NO values were reliable.

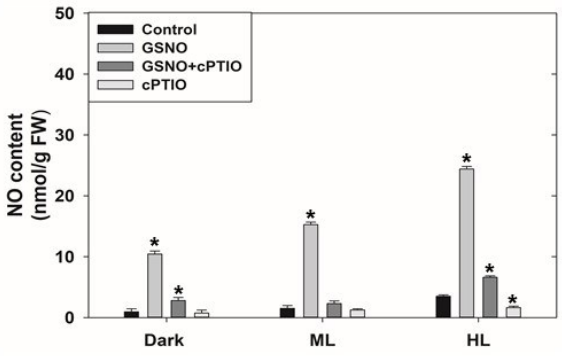

Figure 1. The levels of NO in leaves of pea exposed to GSNO (1 mM) for 3 h under dark, moderate (ML, 300 µE $m^{-2} s^{-1}$), or high light (HL, 1200 µE $m^{-2} s^{-1}$). The values validated the levels in the presence of cPTIO (1 mM), a scavenger of NO. Averages ± SE of 3 experiments on different days and their significance * $P < 0.05$; ** $P < 0.001$ (compared to control, no GSNO).

NO inhibited both photosynthesis and respiration

Incubation with GSNO severely inhibited photosynthesis and respiration in ML and HL, compared to that in the dark (Figure 2). Further, the inhibitory effect of GSNO on respiration was stronger than that of photosynthesis.

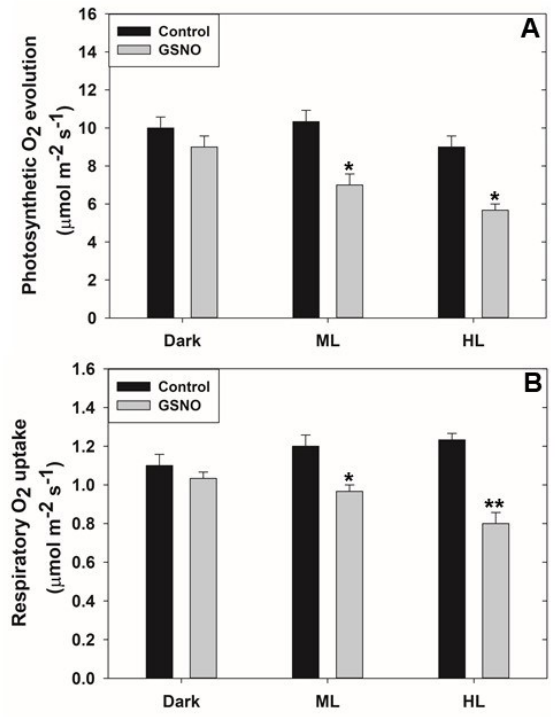

Figure 2. Photosynthetic O_2 evolution (A) and respiratory O_2 uptake (B) after exposure to GSNO (1 mM) under ML or HL for 3 h. Further experimental and statistical details were as in Figure 1.

Effect of NO on photochemical components, as indicated by Chl fluorescence

Most of the PSII related parameters (monitored at 800 µE m^{-2} s^{-1}) [Fv/Fm, ETR(II), Y(II), NPQ, and Y(NPQ)] were all decreased in dark, ML and HL, on treatment with GSNO compared to untreated control (Supplementary Table S1). The decrease in GSNO-treated samples under HL was up to 33 % (Figure 3A–E). Similarly, the Y(NO) values increased by 156 % with GSNO, again under HL (Figure 3F).

Among the PSI-related parameters (monitored at 1000 µE m^{-2} s^{-1}) (Supplementary Table S2), the decrease by GSNO of Pm, ETR(I), and Y(ND) was marginal (Figure 4A-D). However, the Y (NA) decreased due to GSNO under light (ML or HL) (Figure 4E).

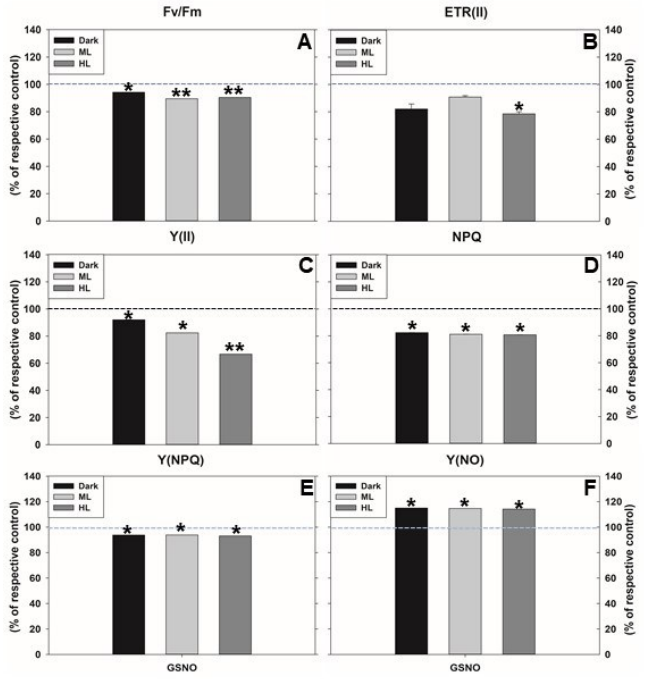

Figure 3. Chl fluorescence-based PSII parameters in leaves after a 3 h exposure to GSNO (1 mM) under dark or ML or HL. The values of PSII-based chl fluorescence parameters were measured at an intensity of 800 µE m^{-2} s^{-1}. Abbreviations used: Fv/Fm-Maximal PSII quantum yield; ETR(II)-Electron transport rate of PSII; Y(II)-Quantum yield of PSII; NPQ- Non-photochemical quenching coefficient; Y(NPQ)-Quantum yield of NPQ-regulated energy dissipation of PSII; Y(NO)- Quantum yield of nonregulated energy dissipation of PSII. Further experimental and statistical details were as in Figure 1.

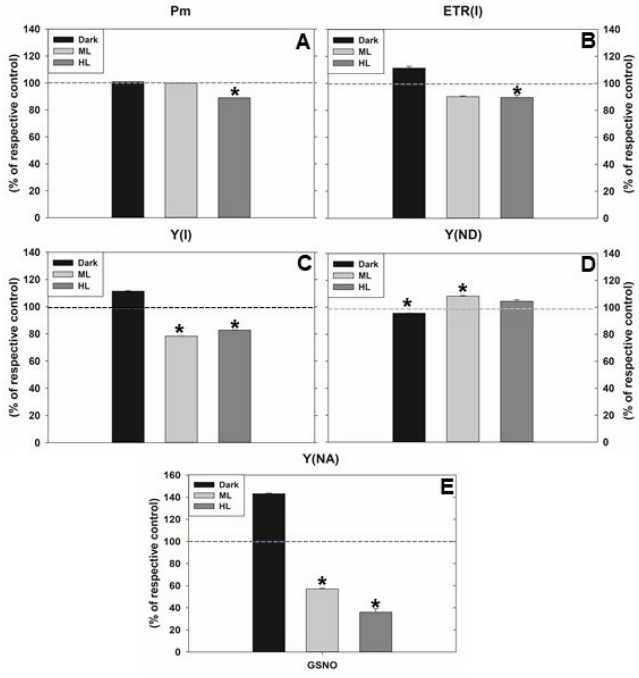

Figure 4. Chl fluorescence-based PSI parameters in leaves after a 3 h exposure to GSNO (1 mM) under dark or ML or HL. The PSI-based chl fluorescence parameters were measured at an intensity of 1000 µE m^{-2} s^{-1}. Abbreviations used: Pm-Maximal P700 change; ETR(I)-Electron transport rate of PSI; Y(I)-Quantum yield of PSI; Y(ND)-Donor side limitation of PSI; Y(NA)-Acceptor side limitation of PSI. Further experimental and statistical details were as in Figure 1.

Discussion

Our results revealed that NO modulated both PSII and PSI. The damage caused to PSII appeared to extend to PSI. The effects of NO were quite pronounced under HL compared to that in darkness or ML.

NO elevation in GSNO-treated leaves (Figure 1) was not surprising. The NO levels were increased by light, particularly HL, even without GSNO, but this increase was amplified further in the presence of GSNO. Thus, illumination intensified the effects of NO-donor. Our observations are similar to the reports on the marked increase in NO levels

on treatment with external NO donors in *Lupines termis, Medicago truncatula* and *Nicotiana tabacum* (25-27).

The suppression of photosynthesis and respiration was moderate in the presence of GSNO (Figure 2). The inhibition by NO of photosynthesis was known (6, 19). But there were only a few studies on the NO effect on respiration (28, 29). We emphasize that both photosynthesis and respiration are sensitive to GSNO. The inhibition of photosynthesis by SNP, another NO-donor, was reported in mesophyll protoplasts and leaves of pea (6, 19, 30). However, the exact mechanism of NO action was still unknown. The sensitivity of photosynthesis was obviously due to the interference by NO of PSII components followed by PSI, as observed by us (6, 19). The sensitivity of respiration could be due to the interference with the cytochrome pathway, as NO and its derivatives caused irreversible inhibition of cytochrome c oxidase (31).

Both the photosystems (PSII and PSI) appeared to be targets of NO. However, PSII was more sensitive than PSI to NO, as indicated by Chl fluorescence parameters (Figure 3A–F). The marked sensitivity of PSII components to NO was previously noted (19, 32). When present, NO either down-regulated PSII-related parameters (13, 17, 33) or up-regulated the PSI-related reactions (14). In our studies, GSNO exerted stronger inhibition on PSII components than on PSI (Figure 3A–F). A high concentration of exogenous NO strongly inhibited the PSII electron transport between Q_A and Q_B (13, 17, 34).

The status of Fv/Fm can be treated as a stress indicator for Chl a fluorescence. In intact potato leaves, SNP reduced the Fv/Fm but did not cause any change in NPQ (18). On the other hand, another NO donor (GSNO) down-regulated Fv/Fm and NPQ in intact pea leaves (17). Based on our observations on PSII parameters (Figure 3A–F), we propose that most reaction centres switched from photochemistry to heat dissipation after treatment with NO, possibly due to structural reorganization in PSII.

The exposure to GSNO under ML or HL caused more damage to PSII/PSI than when kept in darkness. It is possible that the presence of NO aggravated the stress caused by HL and *vice versa* (Figure 3 and 4). A requirement for illumination to ensure NO-mediated damage to PSII in *V. faba* was reported by Ördög *et al.* (35). PSII damage depended on light intensity and the speed of recovery depended on the chloroplast's energetic state (36, 37). Our results endorsed the view that the damage caused by NO was aggravated by light, particularly HL. There were suggestions that NO can be an antioxidant component alleviating oxidative stress caused by HL and providing a photoprotection mechanism (38, 39).

Solymosi *et al.* (40) recently showed that NO released by SNP and GSNO affected the photochemical efficiency of both PSII and PSI. Wodala and Horvath (14) also observed that GSNO-induced NO increased PSI quantum efficiency in intact pea leaves. In our experiments, the marked change in the PSI components with NO reflected a significant difference in PSI photochemistry (Figure 4A–E). The increase in Y(NA) in contrast to decreased Y(ND) under HL indicated the accumulation of electrons on the PSI donor side (Figure 4D, E), as observed by Munekage *et al*. (41) in a mutant of *A. thaliana.* We propose that the observed effects of GSNO: restriction of Y(ND) and stimulation of Y(NA) reflect PSI-based cyclic electron transport stimulation. Our observations endorsed the opinion that PSI was more robust than PSII, and the extent of damage to PSII was severe at HL (1, 37).

Conclusion

In our experiments, GSNO was a good NO-donor, as it could release NO, and affect PSII/PSI with a marginal effect on overall photosynthesis, respiration, and pigments. We endorse the view that NO affects PSII components and restricts electron transport. The reactions of PSI were affected marginally. The effects of NO on PSII or PSI were always quite pronounced in HL. The staining of pea leaves with diaminobenzidine or nitrobluetetrazolium confirmed the elevated levels of ROS in light, particularly HL (Supplementary Table S3). We, therefore, propose that the effects of NO were aggravated in HL, possibly due to endogenous reactive molecules: ROS or NO or both.

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Authors contributions

ASR conceptualized the study. DS performed most of the experiments, while RBB, JP, VA, BS, SG, and PB did some. ASR and DS analyzed the results and wrote the first draft. ASR, DS, and RS further edited the MS. The final manuscript was read and approved by all the authors.

Compliance with ethical standards

Conflict of interest: The authors have no conflicting interests.

Ethical issues: None.

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