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Nitric oxide modifies photosynthetic electron transport in pea leaves

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ABSTRACT Previous electron paramagnetic resonance (EPR) and chlorophyll *a* fluorescence studies on isolated thylakoid membranes showed that nitric oxide (NO), a transmembrane messenger gaseous free radical, slows down the rate of photosynthetic electron transport *in vitro*. NO could reversibly bind to several sites of photosystem II (PS II) (e.g. non-heme iron complex between Q_A and Q_B, Q_B binding site, water-oxidizing complex) by replacing bicarbonate and causes an inhibitory effect on photophosphorylation. Our results show that *in vivo* application of NO by several specific NO donor molecules slowed down the rate of Q_A^- reoxidation in pea leaves. NO reduced the optimal quantum efficiency by increasing the dark fluorescence yield (F_o) and decreasing the variable fluorescence (F_v). It also decreased the photochemical quenching (qP) and modified the non-photochemical (NPQ), mainly energy-dependent quenching (qE) in a concentration related manner. Dark relaxation of NPQ also showed, that NO enlarged the photoinhibitory quenching. Since NO acts as a signalling molecule in plant cells during various stresses, our results predict that NO, in a nanomolar concentration range, can assist to avoid the potential stress induced photodamage by inducing heat dissipation of excess light in the PS II antenna. In contrast, higher, cytotoxic concentrations, NO serves as a photosynthetic inhibitor.
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KEY WORDS

nitric oxide
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Plants not only respond to ambient levels of nitric oxide (NO), but also generate NO by nitric oxide synthase-like isozymes and other abiotic ways. In the past years, this gaseous free radical has been implied as an important signaling molecule in many physiological processes. NO induces elongation in root segments; it also stimulates seed germination and de-etiolation, as well as inhibiting hypocotyl elongation. Recently it has been shown to mediate plant defense responses against pathogens. Nevertheless, the role of NO in photosynthesis is not completely revealed. In previous studies, the regulating activity of NO in photophosphorylation in chloroplasts was clearly demonstrated. NO inhibits electron transport and light-induced pH formation across thylakoid membrane. The NO donor, S-nitroso-*N*-acetylpenicillamine (SNAP) reversibly reduces ATP synthesis (Takahashi and Yamasaki, 2002). Interestingly, the optimal quantum efficiency (F_v/F_m) was not altered by increasing levels of NO in isolated thylakoid membranes, while others showed reduced quantum efficiency in intact potato leaves upon such treatment (Yang et al. 2004). It was found that photochemical quenching (qP) and the effective quantum efficiency (Φ_{PSII}) decreased while non-photochemical quenching (NPQ) remained unchanged following treatment with the NO donor, sodium nitroprusside (SNP). In our study, we focused on the potential effects on photosynthetic electron transport using fast relaxation analysis of flash induced increase and subsequent decay of chlorophyll *a* fluorescence.

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Materials and Methods

Pisum sativum L. cv Rajnai Törpe plants were grown in the greenhouse for 2 weeks in a modified Hoagland solution. The youngest fully expanded leaves were excised and the petioles were submerged in Petri dishes containing distilled water, NO donor molecules and scavenger chemicals with various concentrations. Chlorophyll fluorescence of PS II of pea leaves was measured with a PAM fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Flash induced increase and subsequent decay of chlorophyll fluorescence yield from pea leaf discs were measured by a double-modulation fluorometer (Photon System Instruments, Brno, Czech Republic) in the 150 µs – 100 s time range as described earlier (Vass et al. 1999). Multicomponent deconvolution of the relaxation curves were performed by using a fitting function with two exponential components (fast and middle phase) and one hyperbolic component (slow phase). The fast and middle decay components reflect Q_A^- reoxidation via forward electron transport in PSII centres which did and did not contain, respectively, bound plastoquinone molecule at Q_B site at the time of the flash. The slow phase reflects Q_A^- reoxidation via charge recombination with S_2 state of the water oxidising complex via the $Q_A^-Q_B \leftrightarrow Q_AQ_B^-$ equilibrium.

Results and Discussion

Previous electron paramagnetic resonance (EPR) studies on transition metals in electron transfer components showed

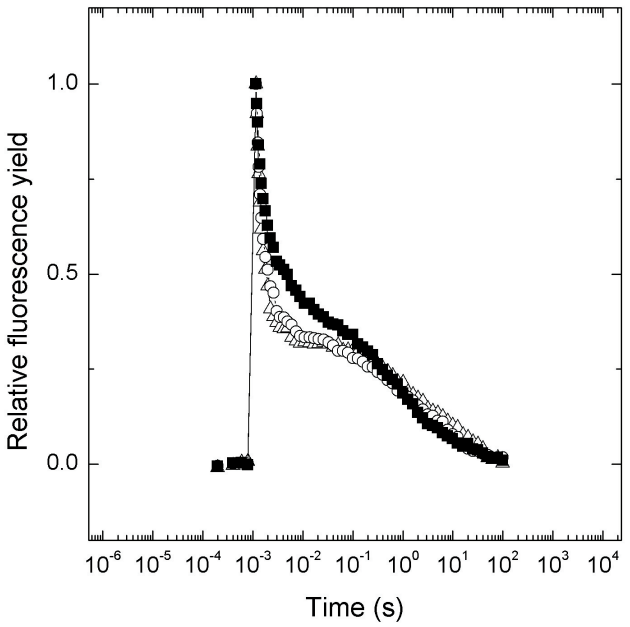

Figure 1. Effect of SNP on fluorescence relaxation of pea leaves. Leaf discs were illuminated for 2 hours at light intensity of 170 µmol m^{-2} s^{-1} in the absence of SNP (open circles), in the presence of 1mM SNP (closed squares) and in the presence of 1mM SNP + 4 g/L haemoglobin (open triangles). Flash induced fluorescence relaxation was measured after 15 min dark adaptation. The curves are normalised to the same amplitude.

that NO can replace bound bicarbonate at different binding sites. NO has a strong affinity to iron in heme and non-heme proteins e.g. the non-heme iron complex in PSII (Diner and Petrouleas 1990). In order to investigate the effect of NO *in vivo*, the Q_A^- reoxidation kinetics in pea leaf discs was measured after 2 hours illumination at light intensity of 170 µmol m^{-2} s^{-1} in the presence of a NO donor, SNP. The increasing concentration of SNP (0.2 mM, 0.5 mM and 1 mM) resulted in slower Q_A^- reoxidation (Fig. 1). The time constants of the fast and middle phase increased in the presence of 1mM SNP compared to control leaves. When haemoglobin, which can bind NO molecule, is present during SNP treatment, no changes can be observed in fluorescence relaxation, indicating that NO and not SNP was an active molecule.

Results above show the *in vivo* effect of NO on Q_A^- reoxidation via forward electron transport in PSII centres both which contained bound plastoquinone molecule and which had an empty Q_B site at the time of the flash. This is in a good agreement with previous data on *in vitro* effect of NO on Q_A to Q_B electron transport (Diner and Petrouleas 1990). Our results indicate that NO displaces the bicarbonate from the acceptor side non-heme iron and as a consequence, slows down the electron transfer between the primary and secondary quinone electron acceptors *in vivo*.

As NO seems to bind to different binding sites of electron transport components, it is not easy to distinguish and identify the individual modulations in the complex chlorophyll *a* slow fluorescence kinetics. F_v/F_m decreases by the increase of SNP concentration due to decline of F_v and increase of F_o levels, which implies a structural alteration of light harvesting complex of PS II. Furthermore, NO abated the photochemical quenching, thus electron transport rate (ETR), tested under different active light intensities.

Raising NO donor concentrations caused an enhancement in non-photochemical quenching, which, in a certain high range, decreased again. Analysis of NPQ recovery indicated that energy-dependent fluorescence quenching (qE) followed the changes in NPQ values, while the photoinhibition component (qI) constantly increased.

These measurements were performed with other specific NO scavengers, cPTIO and haemoglobin, which inhibit the effect of NO in each experiment.

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