

## DISSERTATION SUMMARY

# Combined effects of nitric oxide and cyanide on the photosynthetic electron transport of intact leaves

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Plants not only respond to ambient levels of nitric oxide (NO), but also generate NO themselves (Neill et al. 2003). In the past years, this gaseous free radical has been implicated as an important signaling molecule in numerous physiological processes (Río et al. 2004). Still, the role of NO in photosynthesis is poorly understood, which is well indicated by the modest number of *in vivo* and *in vitro* experiments in this area with mixed results (Takahashi and Yamasaki 2002; Yang et al. 2004). In our study, we aimed to clarify the potential effects of various NO donor molecules on the photosynthetic electron transport in intact leaves by means of quenching analysis of chlorophyll *a* fluorescence.

The youngest fully expanded leaves of 2-week-old *Pisum sativum* L. cv Rajnai Törpe plants 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).  $Q_A^-$  reoxidation kinetics was followed by a double-modulation fluorometer (Photon Systems Instruments, Brno, Czech Republic). Xanthophyll cycle pigments were determined by HPLC.

$Q_A^-$  reoxidation kinetics in pea leaf discs were measured in the presence of the NO donor, sodium nitroprusside (SNP) in order to see whether NO can replace bound bicarbonate at different binding sites, e.g. the non-heme iron complex, in PSII as measured earlier (Petrouleas and Diner 1990). SNP slows down the electron transfer between the primary and secondary quinone electron acceptors *in vivo* in a concentration dependent manner, which indicates that NO displaces the bicarbonate from the acceptor side non-heme iron. Results in the presence of DCMU, which prevents forward electron transport, indicate that NO inhibits charge recombination reactions of  $Q_A^-$  with the  $S_2$  state of the water-oxidising complex, as well as interacting with the tyrosine radical  $Y_D\dot{y}$  in PSII. Applying scavengers haemoglobin and cPTIO

resulted in a complete or partial elimination of these changes, respectively, which indicates a distinct role for NO as well as cyanide in the process.

The joint effect of cyanide and NO – both released by SNP – on photosynthetic processes was observed in the complex chlorophyll *a* slow fluorescence kinetics, and experiments in the presence of the NO-specific scavenger cPTIO allowed clear distinction between effects induced by cyanide or NO. The SNP-induced moderate decrease in  $F_v/F_m$ , an indicator of light harvesting capacity, implies a structural alteration of the light harvesting complex of PS II, and measurements with cPTIO hold cyanide responsible for this concentration dependent decline. Increasing amounts of SNP cause a more dramatic change in quenching parameters  $qP$  and  $NPQ$ , which provide information about the ratio of open reaction centres in PS II (ready for electron transport) and the ratio of absorbed energy dissipated as heat, respectively. Consistent with results from  $Q_A^-$  reoxidation kinetics, dropping  $qP$  values indicate a slowing rate of electron transport – mediated chiefly by NO. Instead of a resulting  $NPQ$  ( $\Delta pH$ ) decrease, the  $\Delta pH$  is increased due to cyanide which inhibits the Calvin cycle, thus proton loss from the lumen. Other measurements with nitrite and nitrate, and another specific NO donor (GSNO) confirm a specific – cyanide-independent – NO effect.

## References

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