

Carina Barth · G. Heinrich Krause

## Study of tobacco transformants to assess the role of chloroplastic NAD(P)H dehydrogenase in photoprotection of photosystems I and II

Received: 22 February 2002 / Accepted: 28 May 2002 / Published online: 21 August 2002  
© Springer-Verlag 2002

**Abstract** *Nicotiana tabacum* L. wild-type plants and transformants ( $\Delta$ ndhCKJ), deficient in functional NAD(P)H dehydrogenase (NDH), were subjected to high light at 20 °C and 4 °C for 2 h to examine a possible role of NDH-mediated cyclic electron flow in protecting photosystems I and II from photoinhibition. Photochemical activity of photosystem I (PSI) was assessed by means of P700 absorbance changes at 810 nm. In addition, potential photosystem II (PSII) efficiency was determined by measuring the ‘dark-adapted’ ratio of variable to maximum chlorophyll fluorescence,  $F_v/F_m$ . Both photosystems were more susceptible to photoinhibition at 4 °C than at 20 °C. However, the degree of photoinhibition was not less in the wild type than in the NDH-deficient plants. To evaluate the efficiency of P700 oxidation in far-red light, a saturation constant,  $K_s$ , was determined, representing the far-red irradiance at which half of the maximum P700 absorbance change was reached. In photoinhibited leaves, a decrease in the efficiency of P700 oxidation (increase in  $K_s$ ) was observed. The increase in  $K_s$  was more pronounced at 4 °C than at 20 °C, but not significantly different between wild-type and  $\Delta$ ndhCKJ plants. Re-reduction kinetics of oxidised P700 in the dark were accelerated to a similar extent in photoinhibited samples of both genotypes and at the two temperatures tested. The data indicate that NDH-mediated cyclic electron flow does not protect PSI against short-term light stress. It is proposed that the observed increase in  $K_s$  represents a protective mechanism that is based on accelerated charge recombination in PSI and facilitates thermal dissipation of excessive light energy.

**Keywords** Chlorophyll fluorescence · Chloroplastic NAD(P)H dehydrogenase · Cyclic electron transport · *Nicotiana* · Photoinhibition · Photosystems I and II

**Abbreviations**  $\Delta A_{810\max}$ : maximum absorbance change of P700 at 810 nm ·  $F_v/F_m$ : ratio of maximum variable to maximum total chlorophyll *a* fluorescence yield ·  $K_s$ : ‘saturation constant’ of photosystem I, i.e. far-red irradiance causing 50% of maximum P700 absorbance change ( $\Delta A_{810\max}/2$ ) · NDH: NAD(P)H dehydrogenase · PAR: photosynthetically active radiation (400–700 nm) · PSI (II): photosystem I (II) · P700: reaction-centre chlorophyll of PSI

### Introduction

Photoinhibition, defined as a decrease in photosynthetic activity induced by light, has been shown to occur under a variety of environmental stress conditions (Powles 1984; Krause 1988, 1994; Aro et al. 1993; Long et al. 1994; Krause et al. 1995; Huner et al. 1996). In most cases, the primary site of photoinhibition has been found to be located in photosystem II (PSII). However, under certain stress conditions photosystem I (PSI) can be inhibited besides PSII, particularly in chilling-sensitive plants at low temperatures. In leaves of chilling-sensitive cucumber and of potato, PSI was observed to be more susceptible to photoinhibition than PSII (Havaux and Davaud 1994; Terashima et al. 1994; Sonoike 1995, 1996a; Barth and Krause 1999). In chilling-sensitive tobacco and pumpkin, high-light exposure at 4 °C resulted in an inhibition of both photosystems to a similar degree (Barth and Krause 1999). Photoinhibition of PSI has also been observed in chilling-tolerant barley (Tjus et al. 1998; Teicher et al. 2000).

Damage to the PSI reaction centre is known to be caused by reactive oxygen species. The iron–sulphur centres ( $F_A$ ,  $F_B$ ,  $F_X$ ) are thought to be the primary targets of reactive oxygen in PSI. Their destruction supposedly triggers proteolysis of the PSI-A/B reaction-

G.H. Krause (✉)  
Institute of Plant Biochemistry,  
Heinrich Heine University Düsseldorf,  
Universitätsstr. 1, 40225 Düsseldorf, Germany  
E-mail: ghkrause@uni-duesseldorf.de  
Fax: +49-211-8113706

C. Barth  
Boyce Thompson Institute for Plant Research at  
Cornell University, Tower Road, Ithaca, NY 14853, USA

centre proteins and of extrinsic polypeptides of the PSI complex (Sonoike et al. 1995, 1997; Sonoike 1996b; Terashima et al. 1998; Tjus et al. 1999).

Cyclic electron transport around PSI, i.e. the light-driven transfer of electrons from the plastoquinone (PQ) pool via PSI and ferredoxin back to the PQ pool (Bendall and Manasse 1995), has been proposed to contribute to photoprotection of PSI and indirectly also of PSII (Heber and Walker 1992; Fork and Herbert 1993; Endo et al. 1999; Teicher et al. 2000). Cyclic electron flow may reduce the transfer of electrons to molecular oxygen (Mehler reaction) and thus the formation of deleterious reactive oxygen species on the reducing side of PSI. Generation of a transthylakoid pH gradient associated with cyclic electron transport may contribute to down-regulation of PSII activity by increased thermal dissipation of excitation energy. Thereby, the flux of electrons from PSII to PSI would be controlled (Demmig-Adams and Adams 1992; Heber and Walker 1992; Van Wijk and Van Hasselt 1993; Sonoike 1996a).

Two principle cyclic electron transport pathways have been suggested (Bendall and Manasse 1995; Teicher and Scheller 1998; Joet et al. 2001). One, sensitive to antimycin A, supposedly uses ferredoxin-plastoquinone oxidoreductase, whereas the other one, insensitive to antimycin A, involves the thylakoid NAD(P)H reductase (NDH) complex (Joet et al. 2001). NDH subunits, encoded by the plastidic genome of higher plants, are homologous to the proton-pumping NADH-ubiquinone-oxidoreductase in the mitochondrial respiratory chain. The NDH complex has been detected in stroma lamellae, i.e. situated close to PSI (Nixon et al. 1989). In bundle sheath chloroplasts of  $C_4$  plants of the NADP/malate dehydrogenase type, which are deficient in PSII, the function of the NDH complex in cyclic electron transport has been proven (Kubicki et al. 1996). To clarify the role of the NDH complex in  $C_3$  plants, tobacco mutants containing disrupted plastid *ndh* genes have been created and studied by several laboratories (Burrows et al. 1998; Kofer et al. 1998; Shikanai et al. 1998; Endo et al. 1999; Horvath et al. 2000). NDH transformants were identified by the absence of a transient 'dark rise' in chlorophyll fluorescence after the end of actinic illumination. This phenomenon has been interpreted to reflect a slow electron donation from NAD(P)H to the PQ pool in the dark (Kofer et al. 1998; Shikanai et al. 1998). These results led to the conclusion that the NDH complex is not only involved in cyclic electron flow around PSI in the light, but also in chlororespiration in the dark (Burrows et al. 1998; Kofer et al. 1998; Shikanai et al. 1998; Joet et al. 2001).

The aim of the present study is to evaluate the possible physiological significance of NDH-mediated cyclic electron flow in photoprotection of PSI. Leaf discs of tobacco transformants with defects in the *ndhC-K-J* operon (in the following designated as  $\Delta ndhCKJ$  plants) and of wild-type plants were subjected to 2,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation (PAR) at 4 °C and at 20 °C. The effects of high-light exposure on

PSI were assayed by measurement of P700 oxidation in far-red light and re-reduction kinetics of P700<sup>+</sup> in the dark. In parallel, potential efficiency of PSII was determined by chlorophyll fluorescence analysis to clarify whether NDH activity may also protect PSII.

## Materials and methods

### Plant material and plant growth

NDH plastid transformants of tobacco (*Nicotiana tabacum* L. cv. Petit Havana) used in this study were generated by inserting a mutant plasmid to delete the *ndhCKJ* genes (Kofer et al. 1998). The plastid transformation was performed by using biolistic technology as reported by Svab and Maliga (1993). The  $\Delta ndhCKJ$  plants used in this study correspond to the mutant series 53 described by Kofer et al. (1998). Tobacco wild-type and  $\Delta ndhCKJ$  plants were kindly provided by S. Kösling and Dr. K. Steinmüller (Institute of Developmental and Molecular Biology of Plants, Heinrich Heine University Düsseldorf, Germany).

Wild-type plants and NDH transformants were grown in the greenhouse at approx. 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR, 10 h/14 h dark/light-cycle. Temperature was 22 °C and relative humidity was 65%. Mature, fully developed leaves of 8- to 9-week-old plants were taken for experiments.

### Photoinhibition

Before photoinhibitory treatment, detached leaves were dark-adapted for 1 h. Leaf discs (1.5 cm<sup>2</sup>) were photoinhibited as described elsewhere (Barth and Krause 1999). At the times given, discs were taken for determination of PSII and PSI activities.

### Chlorophyll fluorescence analyses and measurement of potential PSII efficiency

To verify the mutant phenotype of  $\Delta ndhCKJ$  plants, the lack of the 'dark-rise' in chlorophyll fluorescence after the end of actinic illumination was measured as reported by Kofer et al. (1998).

The ratio of variable to maximum chlorophyll *a* fluorescence ( $F_v/F_m$ ) was used as a measure of potential PSII efficiency.  $F_v/F_m$  ratios were measured after 10 min dark-adaptation as described by Barth and Krause (1999).

### Measurement of PSI activity

After determination of potential PSII efficiency, PSI activity was determined by means of P700 absorbance changes measured at 810 nm in the reflectance mode (Klughammer and Schreiber 1998). Measurements were performed similar to a procedure described by Barth et al. (2001), using a PAM 101/102/103 fluorometer system connected to a dual-wavelength emitter-detector unit ED-P700DW (Walz, Effeltrich, Germany) and a chart recorder (Servogor 320; BBC Goerz, Austria). Saturating far-red light of 16 W m<sup>-2</sup> (uncorrected for wavelength sensitivity of the pyranometer, Li-200SA; Li-Cor, Lincoln, Neb., USA) was provided by two far-red diodes (102-FR; Walz). A KL-150 lamp (Schott, Mainz, Germany) equipped with an RG 9 filter (Schott) was additionally installed in order to check far-red saturation. Temperature was kept at 20 °C with a thermostat (Colora, Lorch, Germany). Before applying far-red irradiation, samples were pre-illuminated with 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  actinic white light for 5 min. Subsequently, the baseline representing the reduced form of P700 was recorded. Then saturating far-red light was applied to trigger photooxidation of P700. The maximum signal difference ( $\Delta A_{810\text{max}}$ ) between the reduced and oxidised states of P700 was taken as a relative measure of the photochemical capacity of PSI.

Half-times ( $t_{1/2}$ ) of P700 oxidation in far-red light and re-reduction kinetics in the dark were determined using far-red light from one photodiode ( $9 \text{ W m}^{-2}$ ). This far-red fluence rate was almost saturating in controls but not in photoinhibited samples. After pre-illumination with moderate white light (see above), the signal of P700 oxidation in far-red light was recorded until steady state was reached (30 s), followed by recording the re-reduction kinetics of  $\text{P700}^+$  in the dark (speed of the chard recorder,  $1 \text{ cm s}^{-1}$ ). Whereas the P700 oxidation is a mono-exponential reaction, the re-reduction of  $\text{P700}^+$  has been shown to be biphasic (Manuel et al 1999; Cornic et al. 2000; Barth et al. 2001). In the present study only the mean half-time of total re-reduction was determined.

From P700 absorbance changes measured as a function of increasing far-red fluence rates, a 'saturation constant',  $K_s$ , was calculated that represents the far-red irradiance at which half of the maximum absorbance change ( $\Delta A_{\text{max}}/2$ ) was reached in the steady state.  $K_s$  was determined as described by Barth et al. (2001) with the modification that increasing far-red fluence rates (1.9, 2.8, 4.1, 6.2, 9.0, 10, 11, 12, 14, 16, 32, 41, 46  $\text{W m}^{-2}$ ) were produced by two far-red diodes (102-FR; Walz) and a KL-150 (Schott) lamp equipped with an RG 9 filter (see above).

## Results

As reported earlier (Kofer et al. 1998; Shikanai et al. 1998), leaves of wild-type tobacco, but not of NDH-deficient plants, exhibited after the end of actinic illumination a transient fluorescence rise lasting several minutes (data not shown). Further phenotypic characteristics of the  $\Delta\text{ndhCKJ}$  plants, compared with the wild type, have been described by Kofer et al. (1998). The growth of  $\Delta\text{ndhCKJ}$  plants was reduced compared with the wild type. In their outer appearance, the mature leaves used for the experiments did not differ between

the wild type and transformants; leaves of  $\Delta\text{ndhCKJ}$  plants did not exhibit any bleaching or lesions.

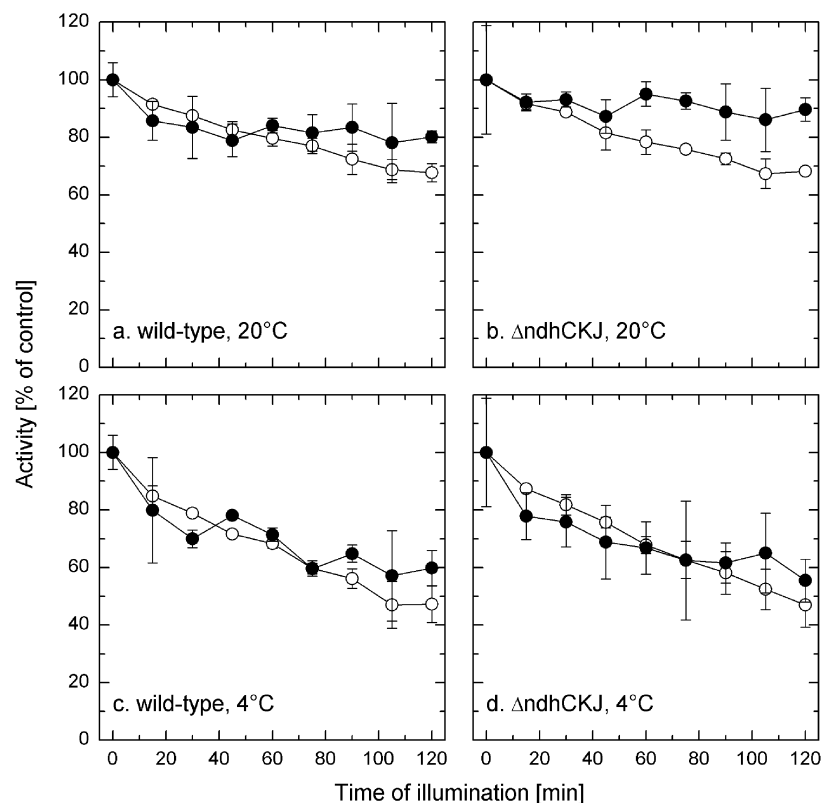
### Effects of photoinhibitory treatment on photochemical capacity of PSI and potential efficiency of PSII

If cyclic electron transport via the NDH complex provides photoprotection,  $\Delta\text{ndhCKJ}$  should show a more pronounced photoinhibition of PSI and possibly of PSII than wild-type plants. However, during 120 min high-light exposure at  $20^\circ\text{C}$  (Fig. 1a, b) and at  $4^\circ\text{C}$  (Fig. 1c, d) no differences in the degree of photoinhibition between the two types of plant were observed. At  $20^\circ\text{C}$ , the photochemical capacity of PSI decreased by about 20% in the wild type and to a slightly, but not significantly, lower extent in  $\Delta\text{ndhCKJ}$  plants. Potential PSII efficiency was diminished by about 30% in both genotypes. At  $4^\circ\text{C}$ , activities of PSII and PSI decreased in parallel by about 40–50% of the control values both in the wild type and  $\Delta\text{ndhCKJ}$ . Contrary to expectation, the data indicate that the presence of a functional NDH complex does not diminish the extent of photoinhibition of PSI or of PSII.

### Effects of photoinhibitory treatment on the saturation constant, $K_s$ , of P700 oxidation

The saturation constant,  $K_s$ , representing the far-red irradiance at which half of the maximum absorbance

**Fig. 1** Effects of photoinhibitory treatment on the photochemical capacity of PSI and the potential efficiency of PSII (denoted 'activities') in leaf discs of tobacco (*Nicotiana tabacum*) wild-type (a, c) and  $\Delta\text{ndhCKJ}$  plants (b, d) exposed to a PAR of  $2,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at  $4^\circ\text{C}$  and at  $20^\circ\text{C}$ . Photochemical capacity of PSI (filled circles,  $\Delta A_{810\text{max}}$ ) and potential efficiency of PSII (open circles,  $F_v/F_m$ ) are illustrated as percent of non-photoinhibited dark-adapted controls. Mean values  $\pm$  SD of three independent experiments are presented (SD not shown where smaller than symbols).  $F_v/F_m$  ratios of controls: wild type,  $0.820 \pm 0.010$ ;  $\Delta\text{ndhCKJ}$ ,  $0.803 \pm 0.018$



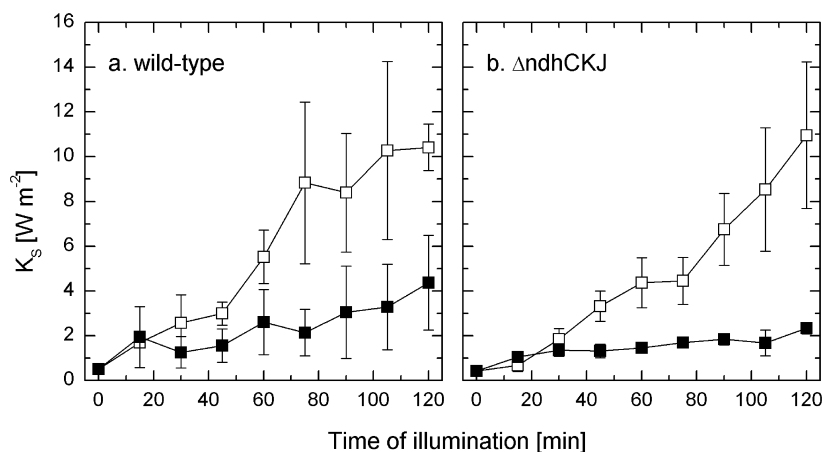
change at 810 nm is reached, was used to assess the efficiency of P700 photooxidation under far-red light that preferentially excites PSI (Barth et al. 2001). Figure 2a, b shows the changes in  $K_s$  in wild-type and NDH-deficient plants caused by irradiation with  $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR at  $20^\circ\text{C}$  and  $4^\circ\text{C}$ . No significant differences in the control values (time 0 min; see also legend to Fig. 2) were observed. Photoinhibitory treatment resulted in an increase in  $K_s$ , i.e. a decrease in the efficiency of P700 oxidation, which was considerably stronger upon treatment at  $4^\circ\text{C}$  than at  $20^\circ\text{C}$ . A tendency towards a stronger  $K_s$  increase in the wild type than in  $\Delta\text{ndhCKJ}$  could be seen, but this difference was not significant. Thus, it appears that cyclic electron transport that is

supposed to be mediated by NDH exerts little or no effect on the efficiency of P700 photooxidation by far-red light.

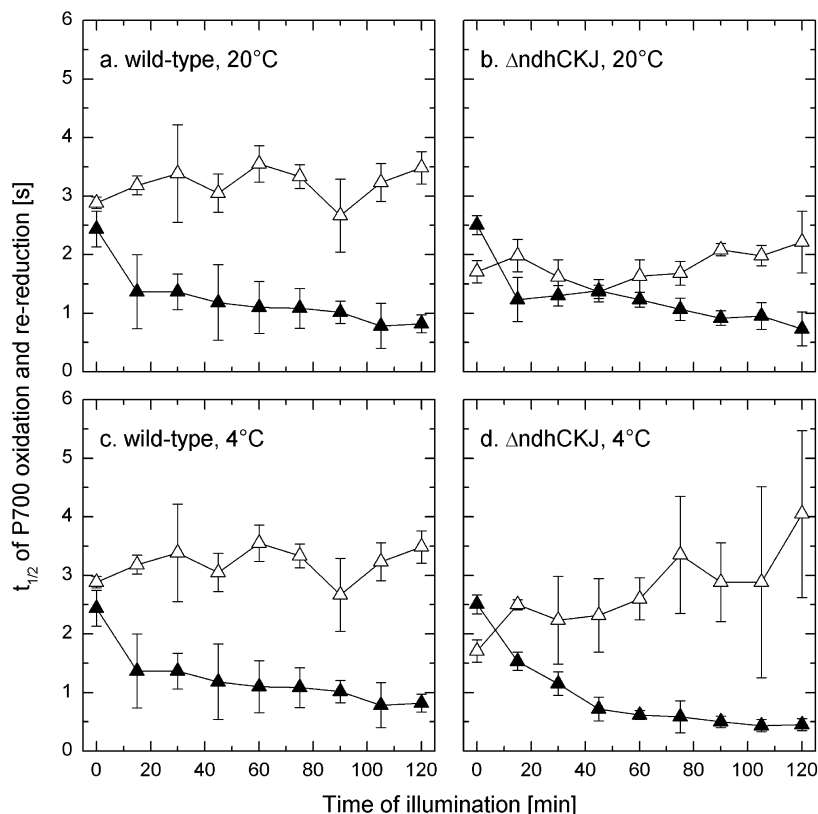
#### Kinetics of P700 oxidation and P700<sup>+</sup> re-reduction

Compared to the wild type, a deficiency in NDH-mediated cyclic electron transfer from PSI to plastoquinone in the transformed plants should result in a faster P700 photooxidation in far-red light, both under photoinhibitory and non-photoinhibitory conditions. Figure 3a–d shows a significant, almost two-times faster P700 oxidation in control leaves of  $\Delta\text{ndhCKJ}$  than in

**Fig. 2** Time course of the saturation constant  $K_s$  of P700 photooxidation under far-red light in leaves of tobacco wild-type (a) and  $\Delta\text{ndhCKJ}$  plants (b). Leaves were subjected to  $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR at  $20^\circ\text{C}$  and  $4^\circ\text{C}$ , respectively. Mean values  $\pm$  SD of three independent experiments are given (SD not shown where smaller than symbols).  $K_s$  values of controls: wild type,  $0.52 \pm 0.04$ ;  $\Delta\text{ndhCKJ}$ ,  $0.42 \pm 0.13$ . Filled squares,  $K_s$  after illumination at  $20^\circ\text{C}$ ; open squares,  $K_s$  after illumination at  $4^\circ\text{C}$



**Fig. 3a–d** Half-times ( $t_{1/2}$ ) of P700 oxidation in far-red light and P700<sup>+</sup> re-reduction in the dark in leaves of tobacco wild-type and  $\Delta\text{ndhCKJ}$  plants. Leaves were irradiated with  $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $20^\circ\text{C}$  (a, b) or at  $4^\circ\text{C}$  (c, d), and subsequently  $t_{1/2}$  was determined. Means  $\pm$  SD of three independent experiments are depicted. Open triangles,  $t_{1/2}$  of P700 photooxidation; filled triangles, mean  $t_{1/2}$  of P700<sup>+</sup> re-reduction



controls of the wild type (compare data points, open symbols at  $t=0$  min), which is a clear indication that NDH-catalysed cyclic electron transport, in fact, takes place in the wild type. Upon photoinhibition at 20 °C,  $t_{1/2}$  of P700 oxidation did not change much, but it increased upon high-light exposure at 4 °C in both genotypes. The latter change seems to reflect the strong increase in  $K_s$  observed after low-temperature photoinhibitory treatment (compare Fig. 2). Mean half-times of P700<sup>+</sup> re-reduction in the dark were identical in controls of the wild type and  $\Delta ndhCKJ$  (about 2.5 s). When exposed to high light at 20 °C or at 4 °C,  $t_{1/2}$  decreased significantly to values below 1 s, indicating an acceleration of P700<sup>+</sup> re-reduction. The decrease in  $t_{1/2}$  did not differ significantly between wild-type and  $\Delta ndhCKJ$  plants, (Fig. 3, closed symbols). These data speak against a substantial contribution of electrons transferred via NDH to P700<sup>+</sup> after the far-red illumination has ceased.

## Discussion

High-light exposure at 4 °C or at 20 °C of leaf discs from tobacco transformants that are disrupted in the *ndhCKJ* genes did not result in enhanced photoinhibition of PSI and PSII compared to the wild type (Fig. 1). These results are in contrast to data reported by Endo et al. (1999). The authors described a significantly accelerated decrease in potential PSII efficiency ( $F_v/F_m$ ) after repeated illumination with strong white light (20 min, 3,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR) in tobacco transformants in which the NDH complex was disrupted by insertional inactivation of the *ndhB* gene. Also, compared to wild type, a lowered photochemical capacity of PSI in far-red light was observed in  $\Delta ndhB$  plants after exposure to strong light. This discrepancy cannot be explained at present, but could be due to an undisclosed side effect of the gene disruption applied by Endo et al. (1999). The authors noted that the measured decreases in  $F_v/F_m$  ratios varied significantly depending on the age of leaves and growth stage of plants.

A saturation constant,  $K_s$ , has been introduced recently as a measure of the efficiency of P700 oxidation in far-red light (Barth et al. 2001). There were only marginal differences between the two genotypes in  $K_s$  values of controls and photoinhibited leaves (Fig. 2). This is consistent with the conclusion of Barth et al. (2001) that the increase in  $K_s$ , as has been observed upon high-light exposure of shade and sun leaves of tropical plants, is not related to PSI-driven cyclic electron transport.

No close correlation between  $K_s$  and P700 oxidation in far-red light or P700<sup>+</sup> re-reduction in the dark (Fig. 3) was found. Wild-type leaves exhibited a substantially slower P700 oxidation than  $\Delta ndhCKJ$  control leaves. That difference can be well explained by an NDH-dependent back flow of electrons to PSI that slows down net P700 oxidation in the wild type, but is lacking in the transformed plants. In the absence of

electron cycling via NDH, electrons might be transferred at enhanced rates from NADPH to acceptors in the chloroplast stroma, such as oxaloacetate (Ivanov et al. 1998).

The increase in the half-time ( $t_{1/2}$ ) of P700 oxidation caused by high-light stress, particularly at 4 °C (Fig. 3c, d), possibly resulted from accelerated cyclic electron transport, which at least in the case of the  $\Delta ndhCKJ$  plants would be NDH-independent. But the  $t_{1/2}$  might also have been influenced by the lowered oxidation efficiency (increase in  $K_s$ , cf. Fig. 2).

The two phases of P700<sup>+</sup> re-reduction (Manuel et al. 1999; Cornic et al. 2000; Barth et al. 2001) have not been resolved here. Mean half-times of re-reduction presented in Fig. 3 were identical for control leaves, and during the course of photoinhibition were decreased in a very similar fashion both at 20 °C and 4 °C in wild-type and  $\Delta ndhCKJ$  plants. In contrast,  $K_s$  values increased much more upon high-light exposure of leaves at 4 °C than at 20 °C, showing a lacking relationship between  $K_s$  and re-reduction kinetics.

Enhancement of P700<sup>+</sup> re-reduction caused by strong pre-illumination of leaves has been suggested to result from accelerated cyclic electron transport (Manuel et al. 1999; Cornic et al. 2000). As no differences in re-reduction kinetics between wild-type and  $\Delta ndhCKJ$  plants could be found, such cyclic electron transfer should be independent of NDH, creating an electron pool that is available in the dark for P700<sup>+</sup> reduction. Moreover, the NDH-mediated electron donation from NAD(P)H to the plastoquinone pool, related to the transient fluorescence rise in the dark (Kofer et al. 1998; Shikanai et al. 1998), appears to be too slow to explain the much faster P700<sup>+</sup> reduction.

Cyclic electron transport could alternatively proceed via the antimycin A-sensitive ferredoxin plastoquinone reductase-mediated pathway, both in wild-type and  $\Delta ndhCKJ$  plants (Shikanai et al. 1998; Joet et al. 2001). PSI has been reported to be very sensitive to photoinhibition in tobacco leaves treated with antimycin A (Chow and Hope 1998), which is consistent with a photoprotective role of such an electron transport cycle.

Taken together, our data suggest that cyclic electron flow via the NDH complex does not contribute to photoprotection of either PSI or PSII under short-term light stress. However, NDH-mediated cyclic electron transport might become important for long-term acclimation to stress conditions. Teicher et al. (2000) reported a substantial up-regulation of the NDH complex in a field-grown winter cultivar of barley in response to photoinhibitory irradiation at low temperatures over a time course of 14 days. Possibly, such up-regulation enhances ATP synthesis, which is required under stress that creates increased need of repair processes.

An acceleration of cyclic electron transport that is independent of NDH was apparently induced by light stress in both genotypes, leading to faster P700<sup>+</sup> re-reduction (Fig. 3). Our data do not exclude that such electron cycling contributes to photoprotection. Lacking

correlation with the increase in  $K_s$  confirms that the latter occurs independently of altered rates of cyclic electron flow. In a previous study (Barth et al. 2001) it was proposed and discussed that the increase in  $K_s$ , which appears to be a ubiquitous response of PSI to light stress, results from charge recombination reactions in the PSI reaction centre based on altered function of FeS centres. Such recombination would dissipate excessive light energy and thereby exert photoprotection. Charge recombination in PSI has also been suggested by Kim et al. (2001) as a response of cucumber leaves to chilling in low light. A further important mechanism of thermal energy dissipation in PSI appears to be the accumulation of a high percentage of P700 in the oxidised state (Barth et al. 2001).

## Conclusion

The study of tobacco transformants in which the NDH complex had been disrupted by insertional mutation of the *ndhCKJ* genes did not reveal evidence that NDH-mediated cyclic electron transport contributes to protection of the photosynthetic apparatus from short-term high-light stress at room or chilling temperature. However, the data presented here and in publications by other authors demonstrate that cyclic electron transport via the NDH complex takes place. The rates may be very low in comparison with the capacity of linear electron transport, but might become of physiological significance under long-term environmental stress. The decreased efficiency of P700 oxidation in far-red light ( $K_s$  increase) caused by light stress may reflect accelerated charge recombination in PSI that facilitates harmless dissipation of excess excitation energy and thus prevents or diminishes severe damage to the PSI complex.

**Acknowledgements** The authors thank Stefan Kösling and Dr. Klaus Steinmüller for providing wild-type tobacco and  $\Delta ndhCKJ$  transformants for experiments. The study was supported by the Deutsche Forschungsgemeinschaft (SFB 189). The paper contains part of the dissertation work of C.B.

## References

- Aro EM, McCaffery S, Anderson JM (1993) Photoinhibition and D1 protein-degradation in peas acclimated to different growth irradiances. *Plant Physiol* 103:835–843
- Barth C, Krause GH (1999) Inhibition of photosystems I and II in chilling-sensitive and chilling-tolerant plants under light and low-temperature stress. *Z Naturforsch C* 54:645–657
- Barth C, Krause GH, Winter K (2001) Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. *Plant Cell Environ* 24:163–176
- Bendall DS, Manasse RS (1995) Cyclic photophosphorylation and electron-transport. *Biochim Biophys Acta* 1229:23–38
- Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. *EMBO J* 17:868–876
- Chow WS, Hope AB (1998) The electrochromic signal, redox reactions in the cytochrome *b<sub>f</sub>* complex and photosystem functionality in photoinhibited tobacco leaf segments. *Aust J Plant Physiol* 25:775–784
- Cornic G, Bukhov NG, Wiese C, Bligny R, Heber U (2000) Flexible coupling between light-dependent electron and vectorial proton transport in illuminated leaves of C-3 plants. Role of photosystem I-dependent proton pumping. *Planta* 210:468–477
- Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599–626
- Endo T, Shikanai T, Takabayashi A, Asada K, Sato F (1999) The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. *FEBS Lett* 457:5–8
- Fork DC, Herbert SK (1993) Electron-transport and photophosphorylation by photosystem-I in-vivo in plants and cyanobacteria. *Photosynth Res* 36:149–168
- Havaux M, Davaud A (1994) Photoinhibition of photosynthesis in chilled potato leaves is not correlated with a loss of photosystem-II activity – preferential inactivation of photosystem-I. *Photosynth Res* 40:75–92
- Heber U, Walker D (1992) Concerning a dual function of coupled cyclic electron-transport in leaves. *Plant Physiol* 100:1621–1626
- Horvath EM, Peter SO, Joet T, Rumeau D, Cournac L, Horvath GV, Kavanagh TA, Schafer C, Peltier G, Medgyesy P (2000) Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. *Plant Physiol* 123:1337–1349
- Huner NPA, Maxwell DP, Gray GR, Savitch LV, Krol M, Ivanov AG, Falk S (1996) Sensing environmental temperature change through imbalances between energy supply and energy consumption: redox state of photosystem II. *Physiol Plant* 98:358–364
- Ivanov B, Kobayashi Y, Bukhov NG, Heber U (1998) Photosystem I-dependent cyclic electron flow in intact spinach chloroplasts: Occurrence, dependence on redox conditions and electron acceptors and inhibition by antimycin A. *Photosynth Res* 57:61–70
- Joet T, Cournac L, Horvath EM, Medgyesy P, Peltier G (2001) Increased sensitivity of photosynthesis to antimycin A induced by inactivation of the chloroplast *ndhB* gene. Evidence for a participation of the NADH-dehydrogenase complex to cyclic electron flow around photosystem I. *Plant Physiol* 125:1919–1929
- Kim SJ, Lee CH, Hope AB, Chow WS (2001) Inhibition of photosystems I and II and enhanced back flow of photosystem I electrons in cucumber leaf discs chilled in the light. *Plant Cell Physiol* 42:842–848
- Klughammer C, Schreiber U (1998) Measuring P700 absorbance changes in the near infrared spectral region with a dual wavelength pulse modulation system. In: Garab G (ed) *Photosynthesis: mechanisms and effects*, vol 5. Kluwer, Dordrecht, pp 4357–4360
- Kofer W, Koop HU, Wanner G, Steinmüller K (1998) Mutagenesis of the genes encoding subunits A, C, H, I, J and K of the plastid NAD(P)H-plastoquinone-oxidoreductase in tobacco by polyethylene glycol-mediated plasmome transformation. *Mol Genet* 258:166–173
- Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiol Plant* 74:566–574
- Krause GH (1994) Photoinhibition induced by low temperatures. In: Baker NR, Bowyer (eds) *Photoinhibition of photosynthesis. From molecular mechanisms to the field*. BIOS Scientific Publishers, Oxford, pp 331–348
- Krause GH, Virgo A, Winter K (1995) High susceptibility to photoinhibition of young leaves of tropical forest trees. *Planta* 197:583–591
- Kubicki A, Funk E, Westhoff P, Steinmüller K (1996) Differential expression of plastome-encoded *ndh* genes in mesophyll and bundle-sheath chloroplasts of the C-4 plant *Sorghum bicolor* indicates that the complex I-homologous NAD(P)H-plastoquinone oxidoreductase is involved in cyclic electron transport. *Planta* 199:276–281

- Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. *Annu Rev Plant Physiol Plant Mol Biol* 45:633–662
- Manuel N, Cornic G, Aubert S, Choler P, Bigny R, Heber U (1999) Protection against photoinhibition in the alpine plant *Geum montanum*. *Oecologia* 119:149–158
- Nixon PJ, Gounaris K, Coomber SA, Hunter CN, Dyer TA, Barber J (1989) *psbG* is not a photosystem 2 gene but may be an *ndh* gene. *J Biol Chem* 264:14129–14135
- Powles SB (1984) Photoinhibition of photosynthesis induced by visible light. *Annu Rev Plant Phys* 35:15–44
- Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998) Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. *Proc Natl Acad Sci USA* 95:9705–9709
- Sonoike K (1995) Selective photoinhibition of photosystem-I in isolated thylakoid membranes from cucumber and spinach. *Plant Cell Physiol* 36:825–830
- Sonoike K (1996a) Photoinhibition of photosystem I: its physiological significance in the chilling sensitivity of plants. *Plant Cell Physiol* 37:239–247
- Sonoike K (1996b) Degradation of *psaB* gene product, the reaction center subunit of photosystem I, is caused during photoinhibition of photosystem I: possible involvement of active oxygen species. *Plant Sci* 115:157–164
- Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem-I iron–sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. *FEBS Lett* 362:235–238
- Sonoike K, Kamo M, Hihara Y, Hiyama T, Enami I (1997) The mechanism of degradation of PsaB protein, a reaction center subunit of photosystem I, upon photoinhibition. *Plant Physiol* 114:159–159
- Svab Z, Maliga P (1993) High-frequency plastid transformation in tobacco by selection for a chimeric *aadA* gene. *Proc Natl Acad Sci USA* 90:913–91
- Teicher HB, Scheller HV (1998) The NAD(P)H dehydrogenase in barley thylakoids is photoactivatable and uses NADPH as well as NADH. *Plant Physiol* 117:525–532
- Teicher BH, Møller BL, Scheller HV (2000) Photoinhibition of photosystem I in field-grown barley (*Hordeum vulgare* L.): induction, recovery and acclimation. *Photosynth Res* 64:53–61
- Terashima I, Funayama S, Sonoike K (1994) The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperatures is photosystem I, not photosystem II. *Planta* 193:300–306
- Terashima I, Noguchi K, Itoh-Nemoto T, Park YM, Kubo A, Tanaka K (1998) The cause of PSI photoinhibition at low temperatures in leaves of *Cucumis sativus*, a chilling-sensitive plant. *Physiol Plant* 103:295–303
- Tjus SE, Møller BL, Scheller HV (1998) Photosystem I is an early target of photoinhibition in barley illuminated at chilling temperatures. *Plant Physiol* 116:755–764
- Tjus SE, Møller BL, Scheller HV (1999) Photoinhibition of photosystem I damages both reaction centre proteins PSI-A and PSI-B and acceptor-side located small photosystem I polypeptides. *Photosynth Res* 60:75–86
- Van Wijk KJ, Van Hasselt PR (1993) Photoinhibition of photosystem II in vivo is preceded by down-regulation through light-induced acidification of the lumen: consequences for the mechanism of photoinhibition in vivo. *Planta* 189:359–368