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

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Tobacco (Nicotiana tabacum var Petit Havana) ndhB-inactivated mutants (ndhB−) obtained by plastid transformation (E.M. Horvath, S.O. Peter, T. Joët, D. Rumeau, L. Cournac, G.V. Horvath, T.A. Kavanagh, C. Schafer, G. Peltier, P. Medgyesy-Horvath [2000] Plant Physiol 123: 1337–1350) were used to study the role of the NADH-dehydrogenase complex (NDH) during photosynthesis and particularly the involvement of this complex in cyclic electron flow around photosystem I (PSI). Photosynthetic activity was determined on leaf discs by measuring CO2 exchange and chlorophyll fluorescence quenchings during a dark-to-light transition. In the absence of treatment, both non-photochemical and photochemical fluorescence quenchings were similar in ndhB− and wild type (WT). When leaf discs were treated with 5 μM antimycin A, an inhibitor of cyclic electron flow around PSI, both quenchings were strongly affected. At steady state, maximum photosynthetic electron transport activity was inhibited by 20% in WT and by 50% in ndhB−. Under non-photorespiratory conditions (2% O2, 2,500 μL L−1 CO2), antimycin A had no effect on photosynthetic activity of WT, whereas a 30% inhibition was observed both on quantum yield of photosynthesis assayed by chlorophyll fluorescence and on CO2 assimilation in ndhB−. The effect of antimycin A on ndhB− could not be mimicked by myxothiazol, an inhibitor of the mitochondrial cytochrome bc1 complex, therefore showing that it is not related to an inhibition of the mitochondrial electron transport chain but rather to an inhibition of cyclic electron flow around PSI. We conclude to the existence of two different pathways of cyclic electron flow operating around PSI in higher plant chloroplasts. One of these pathways, sensitive to antimycin A, probably involves ferredoxin plastoquinone reductase, whereas the other involves the NDH complex. The absence of visible phenotype in ndhB− plants under normal conditions is explained by the complement of these two pathways in the supply of extra-ATP for photosynthesis.

During oxygenic photosynthesis of C3 plants, both photosystem II (PSII) and photosystem I (PSI) cooperate to achieve NADP+ reduction using water as an electron donor and generate a trans-membrane proton gradient driving ATP synthesis. Although NADP+ reduction is recognized to be dependent on the activity of both photosystems through electron transport reactions of the “Z” scheme (Hill and Bendall, 1960; Redding et al., 1999), it has early been reported from studies on isolated thylakoids that ATP could be produced by the sole PSI through cyclic electron transfer reactions (Arnon, 1959). The cyclic electron flow around PSI has been extensively studied in thylakoids and/or chloroplasts of C3 plants (for review, see Fork and Herbert, 1993; Bendall and Manasse, 1995). This mechanism has been suggested to provide ATP for a variety of cellular processes, including stress adaptation (Havaux et al., 1991) and CO2 fixation (Furbank and Horton, 1987; Herbert et al., 1990). During photosynthetic CO2 fixation, both NADPH and ATP are used to regenerate ribulose-1,5-bisphosphate and allow functioning of the photosynthetic carbon reduction cycle (Calvin cycle). In the absence of Q cycle, when one NADPH is produced by linear electron transport reactions, four H+ are released in the lumen. If we consider that translocation of three H+ is required for the synthesis of one ATP (Hangarter and Good, 1982), the ATP to NADPH ratio produced during linear electron transport would be around 1.33. However in C3 plants, the ATP to NADPH ratio required for CO2 fixation has been reported to vary from 1.5 to 1.66, depending on the activity of photorespiration (Osmond, 1981). Insufficient ATP consequently would be synthesized for carbon reduction (Heber and Walker, 1992) and different mechanisms, including cyclic electron flow around PSI, have been proposed to fulfill this function.
tion. A central question is the possible involvement of the Q-cycle, a cyclic electron flow inside the cytochrome (cyt) b$_{6}$/f complex (Mitchell, 1975, 1977) able to translocate additional H$^{+}$ and therefore provide extra ATP. However, the obligatory character or the flexibility of the Q-cycle during CO$_{2}$ fixation remains a matter of debate (Davenport and McCarty, 1984; Ort, 1986; Heber and Walker, 1992; Cramer et al., 1996). Other mechanisms, like cooperation with mitochondrial respiration (Krömer, 1995; Hoefnagel et al., 1998) and Mehler reactions (also known as water-water cycle) (Schreiber and Neubauer, 1990) have also been suggested to re-equilibrate the chloroplastic ATP to NADPH ratio by generating extra-ATP, but their contribution during CO$_{2}$ fixation remains to be established.

Cyclic electron transfer reactions around PSI have been early reported to be inhibited by antimycin A (Tagawa et al., 1963). Most studies concluding to an involvement of cyclic electron flow during photosynthesis in C$_{3}$ plants have been based on the effect of this compound on photosynthetic reactions such as photophosphorylation (Cleland and Bendall, 1992), rereduction of P700$^{+}$ (Scheller, 1996), CO$_{2}$-dependent O$_{2}$ evolution (Furbank and Horton, 1987), $^{14}$CO$_{2}$ fixation (Heber et al., 1978; Woo, 1983), or chlorophyll fluorescence (Ivanov et al., 1998). It was suggested that inhibition of photosynthetic reactions by antimycin A was related to the involvement of an antimycin A-sensitive ferredoxin plastoquinone reductase activity in cyclic reactions (Moss and Bendall, 1984; Cleland and Bendall, 1992). The actual efficiency of cyclic electron flow in vivo during photosynthesis of C$_{3}$ plants is still unclear (Heber et al., 1995a). Photoacoustic measurements, which allow a direct and quantitative measurement of energy storage by cyclic electron flow around PSI in vivo, have been used to show the existence of cyclic electron transfer reactions in C$_{3}$ plants, algae, and cyanobacteria (Herbert et al., 1990). However, until now, this technique failed to show significant cyclic activity in C$_{3}$ plants (Herbert et al., 1990; Malkin et al., 1992). For the unicellular alga Chlamydomonas reinhardtii, Ravenel et al. (1994), by studying the effect of antimycin A and of different inhibitors on photoacoustic measurements, proposed that two pathways are operating in vivo around PSI. One pathway was shown to be sensitive to antimycin A, whereas the other would involve a NAD(P)H dehydrogenase activity (Ravenel et al., 1994). The existence of an antimycin A-insensitive cyclic electron pathways around PSI was also proposed in C$_{3}$ plants from experiments performed in vitro (Hosler and Yocum, 1987; Scheller, 1996).

The plastid genome of higher plants contains genes encoding subunits homologous to the proton-pumping NADH:ubiquinone oxidoreductase, a component of the mitochondrial respiratory chain (Ohyama et al., 1986; Shinozaki et al., 1986). An NADH-dehydrogenase complex (NDH) containing some ndh gene products recently has been purified from pea and barley thylakoid membranes (Sazanov et al., 1998; Quiles et al., 2000). To elucidate the function of the plastidial NDH complex in C$_{3}$ plants, ndh genes were inactivated by chloroplast transformation of tobacco (Nicotiana tabacum var Petit Havana) in different laboratories. Inactivation of ndhB, ndhC, ndhK, and ndhJ genes revealed that the NDH complex is dispensable for plant growth under standard conditions (Burrows et al., 1998; Shikanai et al., 1998; Horvath et al., 2000). The absence of a transient postillumination increase in chlorophyll fluorescence in all NDH-inactivated plastid transformants led to conclude that the NDH complex is involved in the dark reduction of the plastoquinone (PQ) pool, this phenomenon being considered as an after effect of cyclic electron flow around PSI (Burrows et al., 1998; Cournac et al., 1998; Kofer et al., 1998; Shikanai et al., 1998). Horvath et al. (2000) recently reported an enhanced growth retardation in ndhB$^{-}$ inactivated plants when grown under controlled conditions of decreased air humidity. Under such conditions, moderate stomatal closure lowers internal CO$_{2}$ concentration, thus increasing the activity of photorespiration. It was proposed by the authors that the NDH complex is involved, via the activity of cyclic electron flow around PSI, in the production of extra-ATP necessary to fulfill the higher ATP demand occurring under photorespiratory conditions.

The aim of the present work is to further study the physiological function of the plastidal NDH complex in plants. For this purpose, we investigated the effect of antimycin A on ndhB inactivated plants (Horvath et al., 2000). We observe an increased sensitivity to antimycin A of ndhB$^{-}$ mutants, this effect being dependent on the photorespiration rate. We conclude to the existence of two cyclic electron transport pathways operating in vivo around PSI, both of these pathways participating to the supply of extra-ATP for photosynthesis.

**RESULTS**

Chlorophyll fluorescence was measured during dark to light transitions on stripped tobacco leaf discs of WT and ndhB$^{-}$. In the dark, the nonactinic modulated light allows to determine the F$_{0}$ fluorescence level (Fig. 1). Maximal efficiency of PSII was 0.78 ± 0.02 and was similar in both WT and ndhB$^{-}$. Upon illumination (230 μmol photons m$^{-2}$ s$^{-1}$), the chlorophyll fluorescence level transiently increased in both WT and ndhB$^{-}$ and then rapidly decreased due to both photochemical and non-photochemical quenchings. Saturating pulses were used to evaluate photochemical (qP) and non-photochemical (qN) quenching values (Fig. 2). Under illumination, chlorophyll fluorescence induction was similar in WT and ndhB$^{-}$ (Figs. 1A and 2A). However, a significant difference between the WT and ndhB$^{-}$ was observed
when turning the light off (Fig. 1A). In the WT, a transient increase in the fluorescence level was observed before reaching the F0 level, this effect being absent in ndhB−. This confirms recent work reporting that the postillumination fluorescence rise is absent in ndh inactivated mutants (Burrows et al., 1998; Cournac et al., 1998; Kofer et al., 1998; Shikanai et al., 1998). Figure 2A shows that qP rapidly increased during the first 2 min of illumination in both WT and ndhB− before reaching progressively a plateau. On the other hand, qN transiently increased after switching on the light and then decreased to a plateau. No significant differences could be detected in qP and qN values between WT and ndhB−.

After treatment with antimycin A (Fig. 1B) the maximal efficiency of PSII was not altered \( (F_v/F_m = 0.78 \pm 0.02 \text{ in both WT and ndhB−}) \), but the chlorophyll fluorescence transient observed following illumination was strongly affected. The fluorescence level \( F_o \) of ndhB− remained at a higher value than the WT and quite noticeably, saturating pulse induced strong oscillations of \( F_o \) in ndhB− leaves (Fig. 1B). The period of the oscillations was between 20 and 30 s. Quenching analysis was performed during oscillations by illuminating the sample with saturating pulses. Determination of qN and qP values clearly show that oscillations in \( F_o \) (Fig. 3A) are due to changes in qP; qN values remaining remarkably stable (Fig. 3B).

As shown in Figure 2B, the transient increase in qN was suppressed by antimycin A in both WT and ndhB−, and the qN value progressively reached a level close to that measured in Figure 2A. The establishment of qP was delayed by the antimycin A treatment in WT, but qP finally reached a plateau close to that measured in the absence of antimycin A. The effect of antimycin A was more drastic on ndhB−. At steady state, differences between fluorescence induction curves of WT and ndhB− treated by antimycin A (Fig. 1B) were mainly explained by differences in qP values, qN values being less affected (Fig. 2B). Similar effects were observed at high light intensity (1,250 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \); data not shown).

Antimycin A is known to inhibit cyclic electron transport in chloroplasts (Woo, 1983) but is also a potent inhibitor of the cytochrome bc1 complex in mitochondria. To determine whether the effect of

Figure 1. Effect of antimycin A on chlorophyll fluorescence induction curves measured on stripped leaf discs of WT and ndhB− tobacoo plants. A, WT and ndhB− in the absence of treatment. B, WT and ndhB− treated with 5 \( \mu \text{M} \) antimycin A. Light intensity was 230 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) during the actinic light period (shown by a black box on the x axis).

Figure 2. Effect of antimycin A and myxothiazol on photochemical (qP) and non-photochemical (qN) quenchings values during a light to dark transition in WT and ndhB− tobacco. Fluorescence quenchings were measured on stripped leaf discs: A, control; B, treated with 5 \( \mu \text{M} \) antimycin; C, treated with 10 \( \mu \text{M} \) myxothiazol. WT, ○ and ■; ndhB− mutant, ▽ and ▼. Light intensity was 230 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \). Fluorescence levels \( F_o, F_m, F_m’, F_o’, F_v \) were measured during illumination and were used to determine photochemical (qP, black symbols) and non-photochemical (qN, white symbols) quenchings.
antimycin A could be attributed to an effect on chloroplasts or mitochondria, we used myxothiazol, another cytochrome bc1 inhibitor that inhibits the mitochondrial complex by interacting with cytochrome b at a different site (von Jagow and Engel, 1981; Thierbach and Reichenbach, 1981). We found that, in contrast to the effect of antimycin A, myxothiazol had no significant effect on qP and a slight inhibitory effect on qN induction curves, this effect being similar in WT and ndhB2 (Fig. 2C).

The light saturation of the photosynthetic electron transport was determined at steady state (Fig. 4). In the absence of treatment, both WT and ndhB− leaf discs showed similar light saturation curves. In WT, the antimycin A treatment decreased the maximum photosynthetic electron transport activity by approximately 15%. This decrease was much more pronounced in ndhB− leaf discs (approximately 50%). At high-light intensities, the effect of myxothiazol on WT photosynthesis was similar to the effect of antimycin A (approximately 15% inhibition). However, in contrast to antimycin A, myxothiazol did not generate an additional effect on ndhB− leaf discs photosynthesis (Fig. 4B). It is interesting that at low and medium light intensities (less than 500 μmol photons m−2 s−1), myxothiazol had no significant effect on the photosynthetic electron transport activity (Fig. 4B), whereas an inhibitory effect of antimycin A was observed on WT samples under these conditions (Fig. 4A). We checked that under our experimental conditions both antimycin A and myxothiazol inhibited the cyt bc pathway of mitochondrial respiration. Respiration rates were measured as CO2 production in the dark on control leaf discs and on leaf discs treated with respiratory inhibitors (Fig. 5). Antimycin A or myxothiazol alone inhibited the respiration rate by respectively 34% and 16%. Salicyl hydroxamic acid (SHAM), an inhibitor of the alternative oxidase, had almost no effect on respiration. Simultaneous addition of myxothiazol and SHAM or antimycin A and SHAM inhibited the respiration rate by 73% and 83%, respectively, thus showing the participation of the alternative oxidase pathway. Note that when added in the presence of antimycin A, myxothiazol, or alone, SHAM had no effect on chlorophyll fluorescence induction curves (data not shown). As antimycin A and myxothiazol similarly inhibited mitochondrial respiration, we conclude that the additional effect of antimycin A on ndhB− leaf discs compared with the WT is not related to a mitochondrial inhibition but rather linked to the inhibition of a chloroplast process.

The effect of antimycin A on photosynthesis was investigated under different photorespiratory conditions, by simultaneously measuring at steady-state...

Involvement of the NDH Complex in Cyclic Electron Flow around PSI

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In addition to its well-known effect on cyclic electron flow, antimycin A has been reported to affect qN (Oxborough and Horton, 1987; Ivanov et al., 1998). In our experiments, antimycin A induced a significant delay in the establishment of qN, but the steady-state level was virtually not affected and no differences were observed between WT and ndhB− even at high intensities, when qN reaches its maximal values. In antimycin-treated leaves, Fv values at steady state remained higher in mutants than in WT, whereas the qN values were comparable. This higher Fv in mutants was then not related to variations in qN, but attributable to a more reduced state of the plastoquinone pool, indicating a less efficient functioning of electron acceptor reactions after PSI (Calvin cycle, etc.). We conclude that the simultaneous inhibition by antimycin A of cyclic electron flow around PSI and of NDH activity by gene inactivation leads to a reduced ability to use reducing power on the acceptor side of PSI.

Previous studies, based on the disappearance of the transient postillumination rereduction of PQ in ndh-inactivated mutants (Burrows et al., 1998; Cournac et al., 1998; Kofer et al., 1998; Shikanai et al., 1998) or on a decrease of the P700− reduction rate in the dark (Burrows et al., 1998) already concluded to an involvement of the NDH complex in intersystem chain reduction and therefore to its potential implication during cyclic electron transport. It appears from our experiments that the NDH activity is involved in cyclic electron transport together with the antimycin-sensitive pathway. Since photosynthesis is only slightly inhibited by antimycin A in WT, we conclude that the NDH-mediated pathway has a sufficient efficiency to compensate for the antimycin-sensitive pathway to a large extent.

The existence of different cyclic electron pathways around PSI has previously been suggested in the literature. In spinach thylakoids, Hosler and Yocum (1985) reported the insensitivity to antimycin A of cyclic photophosphorylations measured in the presence of ferredoxin and NADP+. Based on photoacoustic measurements performed in vivo in C. reinhardtii cells, Ravenel et al. (1994) observed that antimycin A and N-ethyl-maleimide could inhibit PSI energy storage in vivo when added together, these compounds having no effect when added alone. More recently, based on P700− rereduction measurements performed in barley thylakoids, Scheller (1996) proposed the existence of an antimycin-insensitive cyclic electron transport around PSI. The involvement of the NDH complex in cyclic electron flow in association with other pathways was shown in cyanobacteria (Mi et al., 1992; Yu et al., 1993) and recently suggested in higher plants from in vitro experiments performed on broken chloroplasts (Endo et al., 1998). Based on a differential sensitivity to antimycin A of PQ reduction in the WT and in a ndhB− mutant, these authors concluded to the existence of two pathways, one of them involving the NDH complex. All of the evidences obtained in C3 plants are based on experiments performed on in vitro systems. Our study, performed on leaves, clearly shows the importance of cyclic pathways during photosynthesis in C3 plants in vivo.

Figure 5. Inhibition of tobacco leaf discs respiration by antimycin A (5 μM), myxothiazol (10 μM), and SHAM (0.6 mM). WT tobacco leaf discs were stripped and treated with inhibitors. Respiration rates were measured by following CO2 production in the dark. Values are the average of three independent measurements. Vertical bars represent s.e.

PSII-mediated electron transport activity and CO2 assimilation. In air, relative inhibition by antimycin A of PSI activity was approximately 17% for the WT and 33% for ndhB− leaves (Fig. 6A). Similar effects of antimycin A were observed on CO2 assimilation, although differences between WT and ndhB− appeared less obvious. Under non-photorespiratory conditions (2% [v/v] O2, 2,500 μL L−1 CO2), where the ATP demand is decreased (Osmond, 1981), photosynthetic activity of WT leaves was almost unaffected by antimycin A (less than 3% inhibition at the steady state; Fig. 6B). In contrast, in ndhB− leaves relative inhibition by antimycin A was similar to that observed in air (approximately 25% inhibition).

DISCUSSION

Involvement of the NDH Complex in Cyclic Electron Flow around PSI

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Antimycin A was found to induce strong damped oscillations of the fluorescence yield in response to saturating pulse. This effect, observed in ndhB² mutant leaves but not in WT, was attributed to variations in the redox level of QA, since no variation in qN could be detected during oscillations. Oscillations in chlorophyll fluorescence yield and in O₂ evolution rates have been previously reported to be induced by rapid changes in light intensity or gas composition (Slovacek et al., 1980). They have been proposed to result from an imbalance between ATP production (by either linear or cyclic electron transport) and ATP consumption (by photosynthetic carbon reduction or oxidation cycle) processes occurring in response to rapid changes in light intensity or gas composition (Furbank and Horton, 1987; Horton and Nicholson, 1987; Veljovic et al., 1990). Based on the effect of antimycin A on O₂ evolution and chlorophyll fluorescence observed in barley protoplasts during light transitions, and particularly on the fact that antimycin A increased the frequency of oscillations, it was concluded that cyclic electron flow is involved in the ATP balance during the early phase of illumination (Quick and Horton, 1985; Furbank and Horton, 1987).

In our conditions, inactivation of the NDH complex induced strong oscillations of chlorophyll fluorescence in antimycin A-treated leaves, therefore suggesting that the NDH complex is involved in the supply of ATP for photosynthesis.

The differential effect of antimycin A on WT and ndhB² could be observed using antimycin A concentrations as low as 1 μM (data not shown), whereas optimal effects were obtained at 5 μM. At medium light intensities (less than 500 μmol photons m⁻² s⁻¹) inhibition of photosynthetic electron transport by antimycin A was approximately 15%. The inhibition of photosynthesis by antimycin A measured using in vitro systems such as thylakoids or chloroplasts generally reported in the literature is much more important, generally between 50% and 80% (Tagawa et al., 1963; Mills et al., 1978; Woo, 1983; Moss and Bendall, 1984; Cleland and Bendall, 1992). We observed a much more important inhibition of the photosynthetic electron transport activity in ndhB² plants (approximately 50%). One possible explanation for the hypersensitivity of in vitro systems to antimycin A may be the lability of the NDH complex during organelle isolation procedures (Guedeney et al., 1996; Sazanov et al., 1998) or dysfunctioning of the NDH complex under in vitro conditions. It is interesting that Hosler and Yocum (1985) reported particular conditions where photophosphorylation measured in spinach thylakoids membranes system with ferredoxin and NADP⁺ was not sensitive to antimycin A.

Our experiments highlight the importance of cyclic electron transport during photosynthesis in C₃ plants in vivo. This point has been a matter of debate in the last decade given that direct measurements of cyclic electron flow such as those given by photoacoustic experiments failed to detect any significant activity in C₃ plants (Heber and Walker, 1992; Bendall and Manasse, 1995). Using photoacoustic measurements on peas, Malkin et al. (1992) measured a weak cyclic activity, saturating at low-light intensity. In the same way, based on measurements of P700 rereduction, chlorophyll fluorescence, and light scattering on spinach, Heber et al. (1995a) concluded to low cyclic activity in C₃ plants, mainly restricted to the control of PSII, taking part in the complex machinery that acts to protect the photosynthetic apparatus against photo-inhibition. In contrast, and in line with our present data, a recent study by Cornic et al. (2000) based on P700⁺ rereduction and light scattering measurements, performed on pea and spinach leaves, concluded that cyclic electron flow around PSI participates to the ATP supply during photosynthesis. The apparent discrepancy between conclusions based on photoacoustic measurements and those ob-

![Figure 6](https://plantphysiol.org/articles/125/6/1924/F6f02001.jpg)
tained using antimycin A and/or mutations is an intriguing question that remains to be answered.

**ATP Supply, Cyclic Electron Flow, and Photorespiration**

At low-light intensity and under non-photorespiratory conditions, antimycin A had no significant effect on the steady-state photosynthesis rate of WT. Under such conditions, the ATP to NADPH ratio required for CO₂ fixation is only 1.5 (Osmond, 1981) and the NDH complex pathway likely provides sufficient extra-ATP to reach optimal photosynthesis rates. Under the same conditions, the significant effect of antimycin A observed in the *ndhB*<sup>-</sup> mutant (25% inhibition rate), which was not mimicked by myxothiazol, shows that under the lowest ATP demand, a minimal activity of cyclic electron flow is required. As a consequence, we conclude that the Q-cycle is not able to fully satisfy the ATP demand in these conditions. Under photorespiratory conditions, like in air, needs for extra-ATP are increased (Osmond, 1981). In such conditions, antimycin A significantly inhibited electron transport in WT and an enhanced effect was observed in *ndhB*<sup>-</sup> mutants. This likely reflects the fact that the sole NDH-mediated pathway is unable to fully satisfy the ATP demand. These interpretations are consistent with data previously reported on the same tobacco plants (Endo et al., 1999). These authors reported significant growth retardation when growing *ndhB*<sup>-</sup> plants under CO₂ limitation occurring in response to a moderate water limitation or abscisic acid spraying. These conditions induce stomatal closure and consequently reduce internal CO₂ concentration, thus stimulating the photorespiration rate (Cormic and Briantais, 1991; Lawlor, 1995). We therefore conclude, in agreement with Horvath et al. (2000), that the NDH complex is involved in extra-ATP supply under conditions where photorespiration is high. The NDH complex recently was proposed to be involved in photoprotection (Endo et al., 1999). The sensitivity of a *ndhB*<sup>-</sup> mutant to photo-inhibition was explained by an involvement of the NDH complex in the control of electron flow through PSII, which may be mediated by pH changes. Noticeably, photorespiration has been proposed to protect C<sub>3</sub> plants from photodestruction and to prevent photo-inhibition (Heber et al., 1995b; Kozaki and Takeba, 1996). Therefore, a possible role of the NDH complex in producing extra-ATP necessary to sustain high photorespiration rates should also be considered to explain the higher sensitivity of *ndhB*<sup>-</sup> to photo-inhibition.

**Involvement of Other Mechanisms for Extra-ATP Supply and H<sup>+</sup> Requirement for ATP Synthesis**

One of the central questions concerning the debate about the extra-ATP supply for photosynthesis is how the cytochrome b<sub>6f</sub> complex mediates the oxidation of plastoquinol. In case the Q-cycle would be obligatory during the “Z” scheme, electron shuttled back to the plastoquinone pool through the Mitochondrial Q-cycle would increase the H<sup>+</sup> gradient and in turn form more ATP (Davenport and McCarty, 1984; Rich, 1991). If we consider that three H<sup>+</sup> are needed to synthesize one ATP, there would be no need for other mechanisms to supply extra-ATP for CO₂ fixation (Rich, 1988). However, if the H<sup>+</sup>/ATP ratio is four, as reported by Kobayashi et al. (1995) and Rumberg et al. (1990), other mechanisms of extra ATP supply would be needed. Another thing to consider is that, assuming that both Suc and starch are the predominant end products of photosynthesis, there is an additional cost for CO₂ fixation of 0.17 mol ATP per mol CO₂ fixed, consumed in the formation of glycosidic bonds (Furbank et al., 1990). Results presented here provide evidence that an input from cyclic electron transport is essential to fully satisfy the ATP requirements of C<sub>3</sub> plants. This is in accordance with recent studies of Cornic et al. (2000) concluding to the involvement of both cyclic electron flow around PSI and of the Q-cycle for the supply of ATP.

At high-light intensity, maximum photosynthetic electron transport rates measured in WT were inhibited by approximately 20% indistinctly by antimycin or myxothiazol. This effect, which is clearly related to a mitochondrial inhibition, might be explained by a cooperation between chloroplasts and mitochondria to achieve maximal photosynthetic rates. Two mechanisms of interaction can be proposed to explain such a dependency. In the first one, Gly decarboxylation, occurring in mitochondria during the photosynthetic carbon oxidation cycle (or photorespiration), produces NADH. Inhibition of the mitochondrial respiratory chain, by preventing NADH oxidation, might explain such an inhibition. In the second one, part of reducing equivalents produced in the chloroplast during photosynthesis might be shunted to mitochondria. After mitochondrial conversion to ATP, shuttling back to chloroplasts might participate to re-equilibrate the chloroplastic ATP to NADPH ratio. Such a mechanism was proposed by Kroemer (1995), based on the inhibition of photosynthesis by mitochondrial inhibitors like oligomycin in protoplasts. Whatever the mechanism involved in this interaction, it is interesting to note that the mitochondrial contribution is almost undetectable at low light intensity (below 400 mmol photons m<sup>2</sup> s<sup>-1</sup>). We therefore propose that mitochondrial contribution to ATP supply, if it occurs, acts as an ultimate mechanism, which may be used when other mechanisms such as Q-cycle, cyclic pathways are already fully engaged in ATP production.

**CONCLUSION**

In conclusion, differences in steady-state photosynthetic activities could be observed between WT and
NADPH ratio, thus explaining why needed to re-equilibrate the chloroplastic ATP to cyclic pathway and NDH complex are rate (occurring for instance when stomata grow more slowly than WT in response to a water shortage (Horvath et al., 2000).

**MATERIALS AND METHODS**

**Plant Material and Preparation of Leaf Samples**

Wild-type tobacco (*Nicotiana tabacum* var Petits Havana) and ndhB-inactivated mutants (Horvath et al., 2000) were grown on compost in a phytotron (25°C day/20°C night; 12-h photoperiod) under a light fluence of 350 μmol photons m⁻² s⁻¹ supplied by quartz halogen lamps (HQL-T 400W/2000, Osram, Germany). Plants were watered using a half-diluted nutritive solution (Hoagland and Arnon, 1950). Leaf discs (12-mm diameter) were sampled from 5- to 8-week-old plants. After stripping the lower epidermis, leaf samples were kept in the dark on a moist paper filter in a close Petri dish until use. Stripped tobacco leaf discs were soaked in Petri dishes containing water and inhibitors. Times of incubation were respectively 20 and 90 min for photosynthesis and respiration measurements. Inhibitors were added diluted in methanol (maximal final methanol concentration was 0.5% [v/v]). Control leaf discs were soaked in Petri dishes containing water and methanol.

**Chlorophyll Fluorescence Measurements**

Stripped leaf discs were deposited on a wet filter and placed under a watch glass. Chlorophyll fluorescence was measured using a pulse modulated amplitude fluorometer (PAM-2000, Heinz-Walz, Effeltrich, Germany). The optic fiber of the fluorometer was in contact with the watch glass. Non-actinic modulated light (655-nm maximum emission, 600 Hz) was used to determine the chlorophyll fluorescence level *F₀*. Maximum chlorophyll fluorescence level (*Fₘ*) was measured following a saturating pulse (0.8-s duration) of white light (10,000 μmol photons m⁻² s⁻¹). For determination of qP and qN, leaf discs were exposed to actinic light and pulsed every 60 s by a 10,000-μmol photons m⁻² s⁻¹ saturating pulse (0.8-s duration) according to Quick and Stitt (1989). The maximal efficiency of PSII was determined as *Fₘ*/*Fₘ* (Kitajima and Butler, 1975). Apparent PSII activity under illumination, reflecting the electron transport rate of the photosynthetic chain, was estimated from quantum yield measurement as:

\[
(Fₘ* - F₀)/Fₘ* \times \text{PFD, (Genty et al., 1989)}
\]

**Photosynthetic CO₂ Fixation Measurements**

CO₂ exchange measurements were performed using a LICOR LI-6,262 analyzer in a differential mode on stripped tobacco leaf discs kept on a moist paper filter in a homemade chamber. Chlorophyll fluorescence was measured simultaneously using a PAM-2,000 fluorometer as described above. A LICOR LI-610 portable Dew Point Generator was used to generate moist air (75% relative humidity) at a flow rate of 2 mL s⁻¹. A gas mixer (SEMY Engineering, Montpellier, France) was used to generate gas mixtures with various O₂ and CO₂ concentrations. Unless specified, CO₂ concentration was 350 μL L⁻¹ and O₂ concentration was 20%. O₂ concentration was monitored by an O₂ analyzer OXOR 6 N (MAIHAK, Hamburg, Germany) and CO₂ concentration using an infrared gas analyzer (LI-6,262, LI-COR, Lincoln, NE).

**Respiration Measurements**

Five leaf discs (10-mm diameter) were placed on a wet filter paper in the sample chamber of a close gas circuit connected to a UNOR 6 N (MAIHAK) CO₂ analyzer. Respiration was measured at room temperature (20°C) as the CO₂ production rate in the dark.

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**LITERATURE CITED**


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