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# Post-illumination reduction of plastoquinone pool in Δndh tobacco mutants

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### Introduction

Chloroplastic NAD(P)H dehydrogenase (NDH) of higher plant is homologue of eubacterial and mitochondrial complex I in the respiratory chain. NDH consists of 11 subunits encoded in the plastid genome and of unidentified subunits working for oxidation of NAD(P)H, and has putative NAD(P)H:plastoquinone oxidoredutase activity. Physiological role of NDH is still uncertain. One possible role is participating in cyclic electron flow around PS I. Although Z-scheme produces both ATP and NADPH, cyclic electron flow around PS I is supposed to produce only ATP. Therefore cyclic electron flow around PS I bears the more important role under the stress condition which requires more ATP than normal growth condition. Reverse genetics is the strong tool to analyze physiological function. Using plastid transformation in tobacco plant, several groups including us succeeded in generating Δndh mutants (Burrows et al., 1998; Kofer et al., 1998; Shikanai et al., 1998; Horvath et al., 2000). Their Δndh mutants practically showed same phenotypic traits as wild type under the normal growth condition, but some reports suggested that stress conditions were unfavorable for Δndh mutants (Sazanov et al., 1998; Horvath et al., 2000). We previously reported that short but strong light illumination led Δndh mutants to severer photoinhibition than wild type (Endo et al., 1999). To characterize the mechanism of severe photoinhibition in ΔNDH-mutants, we further studied this phenomenon by means of chlorophyll fluorescence analysis.

### Materials and Methods

*Plants*

Transformed tobacco (*Nicotiana tabacum* cv. Xanthi; ΔndhB) in which *ndh B* gene was insertionally inactivated by the *aadA* chimeric gene and control transformant with non-desructive insertion of the *aadA* chimeric gene between *rbcL* and *accD* (4Y26) were used as reported previously (Shikanai et al., 1998). To compare the Δndh phenotype, another transformed tobacco line of in which an *ndhC,K,J* operon was insertionally inactivated by an *aadA* chimeric gene (ΔndhCKJ) was also used (Takabayashi et al. in preparation).

*Measurement of Chlorophyll Fluorescence*

Chlorophyll fluorescence was measured with a PAM-2000 portable fluorometer (Walz, Effeltrich, Germany). The maximum yield of chlorophyll fluorescence (Fm) was induced by a 1-s pulse of saturating white light. Far-red light (>720 nm, $3Wm^{-2}$ ) from an LED and actinic

light from a Xenon lamp were applied to leaves via fiberoptics connected with an emitter-detector.

## Results and Discussion

Post-illumination increase in steady state fluorescence (Fs) measured under weak light

After the repeated illumination of supra-saturating light (each 4min), ΔF/Fm' measured under low light decreased and ΔndhB showed greater decrease than WT (Fig.1). Fluorescence parameters showed that this decrease was mainly due to Fs increase rather than Fm' decrease.

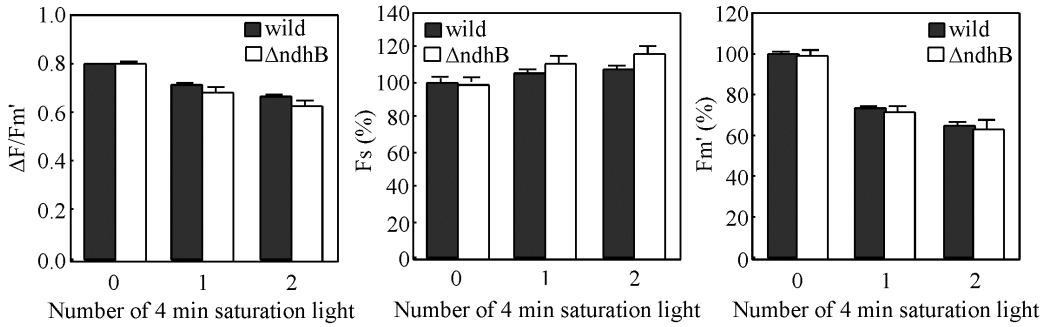


**Fig.1** ΔF/Fm', Fs and Fm' measured under weak light illumination (5 μmol quanta $m^{-2}$ $s^{-1}$) after 4-min illumination of supra-saturating light (3000 μmolE $m^{-2}$ $s^{-1}$) in wild type (WT) and *ndhB* mutant (ΔndhB). Measurements were done 15min after the saturation light was extinguished.

Our previous results suggested that Fs measured under weak light condition (3 μmolE $m^{-2}$ $s^{-1}$) is positively correlated with plastoquinone reduction level (Asada et al., 1993; Endo et al., 1999). Therefore, this larger Fs increase in ΔndhB than in WT suggested that plastoquinone of ΔndhB was more reduced than that of WT after supra-saturating light illumination. On the other hand, this increase in Fs decreased to almost Fo level under far-red light illumination and this effect of far-red light was reversible; i.e., Fs backed to previous level when far-red light was turned off. (data not shown). Far-red light excites reaction center of PS I (P700), but does not excite reaction center of PS II (P680). Therefore almost all plastoquinones are oxidized when far-red light is illuminated under weak light condition. Thus, we considered that Fs decrease under far-red light illumination reflected the changes plastoquinone reduction level. From the above results, we speculated plastoquinone in ΔndhB was more reduced than that in WT after supar-saturating light illumination. This over-reduction of plastoquinone seemed to be somehow contradictory to the fact that ΔndhB lacks the plastoquinone oxidoreductase activity. We are currently hypothesized that Δndh mutants still have plastoquinone reduction activity such as putative ferredoxin-quinone reductase (FQR) (Endo et al., 1998). Further biochemical analysis is conducted to examine following possibility. The higher stromal reduction state or larger amounts of reducing equivalents in stroma of Δndh mutants than WT might induce the reduction of plastoquinone.

*Water stress*

Figure 2 shows that increase of Fs measured under weak light condition (3μmolE $m^{-2}$ $s^{-1}$) after strong light illumination (1200 μmolE $m^{-2}$ $s^{-1}$) in ΔndhCKJ was more increased under water stressed condition than 4Y26 (vector control).

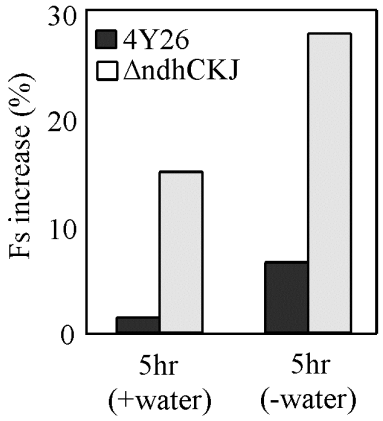


**Fig.2** Post-illumination increase in Fs was more evident under water stress condition. Fs increase after 4min saturation light (1200 μmolE $m^{-2}$ $s^{-1}$ ) was measured in detached leaves with or without water for 5 hours under 25°C under 100 μmolE $m^{-2}$ $s^{-1}$ for 5 hours. Fs was measured under 3 μmolE $m^{-2}$ $s^{-1}$ light.

This increased Fs level was again returned almost Fo level when far-red light was illuminated (data not shown). Similar result was also obtained in both ΔndhB and ΔndhKJ (data not shown). These data suggested that plastoquinone in Δndh mutants was more reduced under dry condition. Our current model to explain this high over-reduction of plastoquinone under dry condition is follows; under dry condition, stomata close and $CO_2$ availability decreases, resulting the decrease of the Calvin cycle and increase of photoinhibition.

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## Reference

Asada K, Heber U, Schreiber U (1993) *Plant Cell Physiol*. **34**:39-50
Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) *EMBO J.* **17**(4):868-76
Endo T, Shikanai T, Sato F, Asada K (1998) *Plant Cell Physiol* **39**:1226-1231
Endo T, Shikanai T, Takabayashi A, Asada K, Sato F (1999) *FEBS Lett.* **457**(1):5-8

Horvath EM, Peter SO, Joët T, Rumeau D, Cournac L, Horvath GV, Kavanagh TA, Schäfer C, Peltier G, Medgyesy P (2000) *Plant Physiol.* **123**(4):1337-50

Kofer W, Koop H-U, Wanner G, Steinmüller K (1998) *Mol Gen Genet.* **258**:166-173
Sazanov LA, Burrows PA, Nixon PJ (1998) *FEBS Lett.* **429**:115-8
Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998) *Proc. Natl. Acad. Sci. USA*, **95**:9705-9708