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# Increased internal resistance to CO<sub>2</sub> diffusion impairs *Nicotiana sylvestris* mitochondrial mutant acclimation to growth light irradiance.



Priault, P.<sup>1</sup>, Tcherkez, G.<sup>1</sup>, Cornic, G.<sup>1</sup>, De Paepe, R.<sup>2</sup> and Streb, P.<sup>1</sup>

<sup>1</sup> Laboratoire Ecologie Systématique Evolution, UMR 8079, UFR Scientifique d'Orsay, Université Paris XI, bât. 362, 91405 Orsay cedex, France.

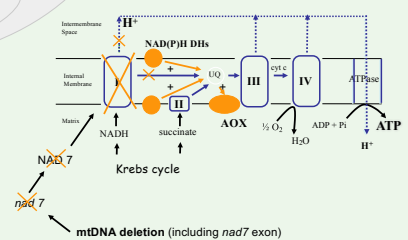
<sup>2</sup> Laboratoire Mitochondries et Métabolisme, Institut de Biotechnologie des Plantes, Université Paris XI, bât. 630, 91405 Orsay cedex, France.

priault.pierrick@ese.u-psud.fr

The CMS II mutant of *Nicotiana sylvestris* lacking the complex I of the mitochondrial electron transport chain (1) is impaired in mitochondria/chloroplasts crosstalks as highlighted by lower net carbon assimilation rates under ambient CO<sub>2</sub> and O<sub>2</sub> conditions and higher dark respiration rates. As mitochondrial function interacts with photosynthetic metabolism, this mutant was used as a tool to investigate the importance of mitochondria in photosynthetic apparatus acclimation to growth light irradiance.

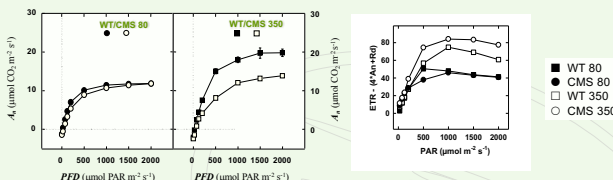
Plants were grown under two irradiances: 80 (LL) and 350 (HL) μmol PAR m<sup>-2</sup> s<sup>-1</sup>. Our first results demonstrated an impairment in net carbon assimilation and initial Rubisco activity in HL-grown CMSII plants as compared to HL-grown WT in spite of equivalent total chloroplastic electron transport and enzyme capacity in both plants (2). The questions raised about the reasons of such impaired acclimation observed in HL-CMSII plants as compared to HL-WT plants and about the fate of the excess electrons, not used for carbon assimilation, in HL-CMSII plants (3).

Respiratory chain of CMS II mutant of *Nicotiana sylvestris*



## Mutant leaves are not able to acclimate photosynthesis to higher growth light irradiance (2).

Investigations of light responses curves of carbon net assimilation in ambient air. The different curves represent low light (LL) and high light (HL) grown CMS and WT leaves (LL=80 μmol m<sup>-2</sup> s<sup>-1</sup>: WT 80, CMS 80 and HL=350 μmol m<sup>-2</sup> s<sup>-1</sup>: WT 350, CMS 350).



The differences in net carbon assimilation previously observed between WT and CMSII plants depend on growth light irradiance and disappear under low light (80 μmol PAR m<sup>-2</sup> s<sup>-1</sup>).

Mitochondrial function is involved in net carbon assimilation adjustment to a higher growth light irradiance.

Our first results show that HL-CMSII leaves are not affected in primary photochemistry (ETR) but have lower Rubisco and SPS activities.

Lower A<sub>n</sub> but similar ETR

Where go excess electrons in HL-CMSII plants ?

The mismatch of ETR, carbon assimilation and initial activity of Rubisco is further investigated in HL-CMSII leaves to characterize alternative electron sinks and identify limiting steps for light acclimation in HL-CMSII plants.

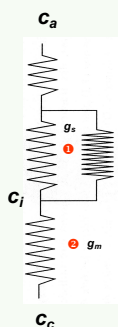
### 1 Oxygen as the major sink of chloroplastic electrons in excess in HL-CMSII plants ?

Net carbon assimilation differences between HL-WT and CMS plants disappear under non-photorespiratory conditions (high light, high CO<sub>2</sub> and low O<sub>2</sub>)

### 2 CO<sub>2</sub> diffusion limitations : stomatal and/or internal ?

Despite a lower stomatal conductance in HL-CMSII plants as compared to HL-WT, for a same external CO<sub>2</sub> concentration, HL-WT and HL-CMSII plants show similar intercellular CO<sub>2</sub> concentration.

In accordance with calculations from models, experimental determinations of chloroplastic CO<sub>2</sub> concentration (C<sub>i</sub>) by coupling isotopic discrimination and gas exchange measurements reveal a two times lower g<sub>m</sub> in HL-CMSII than in HL-WT. Under the equations are represented potential discriminating steps during CO<sub>2</sub> diffusion from the atmosphere to the chloroplasts and during CO<sub>2</sub> fixation by Rubisco, first, considering Farquhar's model (1982) and second considering experimental determination.



$$A_{(i)} = a \cdot (c_a - c_i) / c_a + b \cdot (c_i / c_a)$$

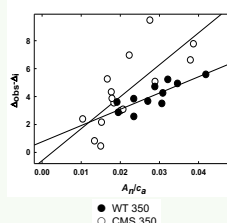
stomata                      Rubisco

$$A_{(i)} = a \cdot (c_a - c_i) / c_a + a_i \cdot (c_i - c_c) / c_i + b \cdot c_c / c_a - d$$

stomata                      Internal conductance                      Rubisco                      Photoresp Resp

$$\Delta_{(i)} - \Delta_{(c)} = (b - a) \cdot (g_m / g_s) \cdot A_{(i)} / c_a + d$$

as: A<sub>i</sub> = g<sub>s</sub> · (c<sub>a</sub> - c<sub>i</sub>) = g<sub>m</sub> · (c<sub>i</sub> - c<sub>c</sub>).



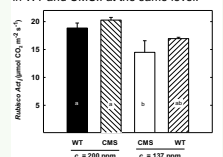
This suggests higher photorespiration rates in HL-CMSII plants as compared to HL-WT confirmed by higher Glycolate Oxidase activity in HL-CMSII plants. Both plants show a similar apparent specificity factor of Rubisco, suggesting a lower CO<sub>2</sub> concentration in HL-CMSII chloroplasts, so a limitation at the level of CO<sub>2</sub> diffusion from the atmosphere to the chloroplasts. → See 2

### 3 Experiments performed to confirm the involvement of CO<sub>2</sub> diffusion limitations in CMSII responses.

#### A Long term growth under high CO<sub>2</sub>



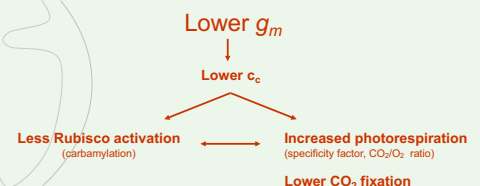
#### B To adjust chloroplastic CO<sub>2</sub> concentration in WT and CMSII at the same level.



Higher CO<sub>2</sub> exposures allow HL-CMSII leaves to recover at least in part its differences comparing HL-WT plants, in terms of net carbon assimilation, growth rate and Rubisco activation, confirming the involvement of the CO<sub>2</sub> supply for Rubisco activity in the impaired acclimation of HL-CMSII leaves.

These results show that mitochondrial function is necessary for photosynthetic acclimation and CO<sub>2</sub> internal diffusion determination to growth light irradiance in *Nicotiana sylvestris*.

Indeed, CMSII plants do not acclimate to higher growth light irradiance as the WT (2). This is shown by a lower net CO<sub>2</sub> assimilation under ambient air conditions and lower activation state of photosynthetic enzymes (2). These differences disappear after an exposure to an atmosphere either enriched in CO<sub>2</sub> and/or depleted in O<sub>2</sub>, or when plants are grown under low-light or high CO<sub>2</sub> conditions. Our results show a lower leaf internal conductance for CO<sub>2</sub> which limits CO<sub>2</sub> supply to the carboxylase activity of Rubisco. This could explain the lower net CO<sub>2</sub> assimilation rates, the lower initial Rubisco activity and higher rates of photorespiration in HL-CMSII as compared to WT, photorespiration constituting the major sink of excess chloroplastic electrons non used for net carbon assimilation in HL-CMSII plants.



References: (1) Gutierrez et al., 1997 (PNAS: 94, pp. 3436-3441)

(2) Priault, P., Fresneau, C., Noctor, G., De Paepe, R., Cornic, G. and P. Streb (2006) The mitochondrial CMSII mutation of *Nicotiana sylvestris* impairs acclimation to growth irradiance. J. Exp. Bot. 57 pp. 2075-2085.

(3) Priault, P., Tcherkez, G., Cornic, G., De Paepe, R., Naik, R., Ghoshghaie, J. and Streb, P. (2006) The lack of mitochondrial complex I in a CMSII mutant of *Nicotiana sylvestris* increases photorespiration through an increased internal resistance to CO<sub>2</sub> diffusion. J. Exp. Bot. (in press).